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Translation

ANTHRAX

By

P.N. Burgasov, et al.

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ANTHRAX

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CONTENTS

Abstract 1

Introduction 2

Brief Historical Information 5

Characteristics of the Pathogen 8

Geographic Distribution 14

Epizootiology 16

Epidemiology 22

Immunology 28

Pathogenesis and Pathological Anatomy 33

Symptomatology 38

Diagnosis and Differential Diagnostics 43

Treatment 47

Laboratory Diagnostics 54

Detection of Pathogen in the Environment 59

Prevention and Control Measures 66

Possible Use of B. Anthracis for Bacteriological Warfare 80

Bibliography 86

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ABSTRACT

This manual, which is intended for a wide circle of practicing physicians, submits current epidemiological and epizootiological information about anthrax. This book deals with questions of pathogenesis, pathological anatomy and symptomatology of this disease.

Some place is devoted in the monograph to differential diagnostics, new methods of diagnosing anthrax, including those involving the use of the allergen, anthraxin. Methods are described for labeling the pathogen of anthrax in the environment by means of bacteriophage and others.

Rational complex methods of therapy are proposed for the treatment of victims of anthrax, with the use of antibiotics, globulin and other drugs.

This book discusses modern prevention and measures to control anthrax, as well as the possibility of using B. anthracis for bacteriological warfare.

INTRODUCTION

Anthrax [called Siberian ulcer in Russian] (anthrax in Latin and English; pustula maligna in Latin; malignant anthrax in English; milzbrand in German; charbon, anthrax charbon, charbon bacteridien in French) is an acute infectious disease in the zoonosis group. Due to the predominant localization of primary lesions, anthrax in man is classified with infections of the integument.

The advances in Soviet health care in the area of lowering and eradicating infectious morbidity are graphically illustrated by the achievements referable to the control of anthrax. In the years of Soviet power, the incidence of this infection, which was a menace at one time, has been lowered to the sporadic level.

In prerevolutionary Russia, anthrax was one of the widespread and dangerous infectious diseases. A huge number of farm animals died annually from it and people were stricken on a mass scale. In 1964, in the European part of Russia alone, more than 90,000 animals died of anthrax; in 1875, about 100,000 horses died in Siberia and in 1879 125,000 sheep fell at one of the sheep farms of southern Russia. There was an average of about 200 cases of anthrax among people for every 10,000 stricken animals.

Introduction in Russia of inoculation of animals against anthrax (1883) lowered somewhat the loss of cattle and morbidity among humans; however, some huge epizootic outbreaks and epidemics of this disease were observed in the nation in subsequent years as well. According to the data of N. A. Mikhin (1942), in 1900-1912, in Russia 40,000-60,000 head of cattle were stricken with anthrax annually, and the total number of anthrax cases among farm animals was recorded at 623,500 (Table 1).

However, it must be borne in mind that such information does not reflect the entire incidence of antrhax in prerevolutionary Russia. According to the data of A. G. Revnivykh (1946), 100,000 deer died of anthrax in in the Bol'shezeml'skaya, Malozemel'skaya, Obskaya, Taymyrskaya and Yeniseyskaya tundras in 1896, 200,000 died in 1897 and 1907, 210,000 in 1911, etc. A total of 1,514,500 deer died of anthrax in the tundras in the period between 1848 and 1917.

Anthrax was recorded among humans in 57 out of the 60 provinces of tsarist Russia. According to N. N. Mari (1916), 268,000 people, 25% of whom died, were stricken in Russia between 1896 and 1913.

According to the far from complete data of the medical department of the Ministry of Internal Affairs, 15,000-20,000 people were stricken annually with anthrax in 1900-1914 (Table 2).

The anthrax-control measures undertaken by the tsarist government were extremely inadequate. Local authorities issued decrees dealing with the control of anthrax from case to case, usually only at the height of mass scale epizootic outbreaks. These decrees were implemented with great delay and poorly. Poverty and ignorance of the people favored the spread of anthrax. The peasants often kept cattle in their dwellings, and this caused them to be infected by sick animals. The carcasses of animals were left in the pastures, which led to occurrence of permanently infected territories. Failure to adhere to elementary standards of labor safety and personal hygiene at enterprises dealing with the processing of raw material of animal origin caused mass scale professional and industrial infection with anthrax. In 1891, V. Svyatlovskiy wrote: "Antrhax exists permanently in Russia. This is our self-styled national disease...."

Table 1. Incidence of anthrax among animals in prerevolutionary Russia, 1900-1912

Year	1900	1901	1902	1903	1904	1905	1906	1907	1908	1909	1910	1911	1912
Number of stricken animals (thousands)	46.6	67.7	54.5	58.6	46.0	52.7	60.4	49.2	42.4	41.2	51.2	44.0	43.2

Table 2. Incidence of anthrax among humans in prerevolutionary Russia

Year	1900	1901	1902	1903	1904	1905	1906	1907	1908	1909	1910	1911	1912	1913	1914
Number of cases (thousands)	11.6	13.9	15.8	17.5	11.9	14.3	18.0	18.1	17.3	19.8	17.8	17.0	15.5	15.2	14.7

The anthrax situation in the USSR changed drastically in the years of Soviet power (Table 3).

In 1966, human morbidity referable to anthrax decreased by 21.5 times, as compared to 1913 and by 2.5 times, as compared to 1950. This was aided by eradication of capitalistic forms of industrial production, socialist reorganization of agriculture, development of veterinary and sanitary inspection [surveillance] and implementation of scheduled preventive measures. In the last few decades, the armamentarium of preventive, diagnostic and therapeutic agents against anthrax in our country was enriched with domestic, highly effective anthrax vaccine, STI. A diagnostic product was developed, "anthraxin," as well as new methods and nutrient media for laboratory diagnosis of anthrax and identification of the pathogen, Soviet strains of indicator bacteriophage; highly effective therapeutic agents were found (anthracic globulin, antibiotics), and new ways and means of disinfection appeared.

Table 3. Incidence of anthrax among the people of the USSR*

Year	Recorded cases		Year	Recorded cases	
	total	per 100,000 population		total	per 100,000 population
1950	1 806	1.00	1958	942	0.45
1951	1 708	0,93	1959	1 016	0.48
1952	1 575	0,84	1960	738	0.35
1953	1 279	0,67	1961	877	0.40
1954	1 236	0,64	1962	846	0.38
1955	1 306	0,66	1963	730	0.33
1956	1 184	0,59	1964	607	0.27
1957	1 293	0,63	1965	673	0.29
			1966	944	0.40

*See the statistical manual, "Human Infectious Diseases in the USSR," edited by P. N. Burgasov, deputy USSR minister of health, Izd. Meditsina, Moscow, 1968.

There is no question but that the broad and purposeful implementation of the existing system of medical anthrax-control measures is capable of further lowering morbidity among humans. At the present time, there is a very real possibility of eradicating occupational-agricultural and occupational-industrial cases of the disease, lowering the incidence to isolated cases arising in rural areas, of eradicating mortality due to anthrax.

In view of the fact that supervision of implementation of veterinary and sanitary measures to prevent anthrax among farm animals was entrusted to public health agencies, activation of the medical service in this direction will be instrumental in drastically lowering morbidity among animals and, consequently, will reduce human morbidity to isolated cases.

In this book, which is intended for a broad circle of practicing physicians, there will be discussion of etiology, immunology, epizootiology and epidemiology of anthrax. Considerable place is devoted to laboratory diagnostics, prevention and measures to control the infection. There is also discussion of current conceptions of the pathogenesis, symptomatological distinctions and rational therapy of anthrax.

The authors will be sincerely grateful for any critical comments and wishes.

BRIEF HISTORICAL INFORMATION

Anthrax was known to mankind in antiquity. Already in the Bible ("Exodus," "Sixth Curse") there was a description of deaths among horses, donkeys, camels, asses and goats due to a disease that resembled anthrax, as well as deaths of people "from evil black spots [murrain]" (quoted from Sobernheim, 1931).

Ancient Greek and Roman writers knew of anthrax under the name of "sacred fire" (ignis sacer), while ancient Arabian physicians called it "Persian fire" (ignis persicus). According to the description of Homer ("Illiad," Bk 1), there were cases of anthrax among mules in the Greek army, during the sieg of Troy, as well as people. Similar information can be found in the works of Ovid ("Metamorphoses," VII). Virgil (Georgics") observed that "it was impossible to remove the hides of dead (oxen), because they could not be cleaned with either air or fire. It was impossible to shear sheep and use their wool infected with poison, or even to touch this spoiled wool" (quoted from V. A. Bashenin, 1955). The historian Pliny the Elder testifies that in the first century AD anthrax was widespread in Narbonnian Gaule (southern France), from which it was carried to Italy in the year 164. Two Roman consuls died of this disease.

In the following centuries, anthrax struck more and more countries and continents. In 896 and 922, deaths among cattle, sheep and swine were observed in all European countries. There were major epizootics of anthrax in Germany in 1375-1376. In the 17th century, epizootic outbreaks of anthrax struck Europe and Siberia (L. V. Gromashevskiy and G. M. Vayndrakh, 1947). According to the data of Afanasiy Kirsher (1658), about 60,000 people died of anthrax in Central Europe, in 1607 (Sobernheim, 1931). There was a major epidemic of anthrax in Moscow in 1649, in connection with which Tsar Aleksey Mikhaylovich issued an ukase forbidding the dumping of animal carcasses on the streets of Moscow (A. I. Metelkin, 1960).

Academician Iogan Gmelin (1767) of Petersburg made the first scientific description of anthrax in Russia; he observed cases thereof among people and cattle during his travels in Siberia in the 1730's. In 1755-1760, A. Eshke and N. G. Nozhevshchik, physicians in the plants of Kolyvano-Voskresenskiy in the Altay area, described the symptoms of anthrax, which made it possible to single this disease out as an independent nosological form of carbunculous lesions in man and animals. In the foreign literature, the French author, Morand, made the first scientific description of anthrax in his paper about this disease delivered in 1766 at the Academy of Sciences in Paris.

The Russian physician, S. S. Andreyevskiy (1760-1818), who was the first to prove that anthrax of man and animals is the same disease, as well as its infective properties by means of self-infection experiments, made a major contribution to the study of anthrax. In 1789, he wrote: "A reliable, tangible experiment was needed, one that would not be questionable, and I performed such an experiment on myself, by inoculating anthrax" (quoted from B. N. Palkin, 1951). S. S. Andreyevskiy described the pathoanatomic findings of the disease, its various clinical forms, and he developed methods of treatment and prevention. The name of S. S. Andreyevskiy is linked with the modern definition of the disease, which he called Siberian ulcer because of the place where it was studied, as reflected in his papers, "On Siberian Ulcer" (1788, 1789) submitted to the Academy of Sciences.

In subsequent years, the Russian physicians I. Peterson (1790) and M. Gamaleya (1792) pointed to the possible role of insects in the spread of anthrax. S. F. Khotovitskiy (1831) studied the geographic distribution of this infection. The epidemiologist, R. S. Chetyrkin (1881) was the first to describe the enteric form of anthrax in man, and he demonstrated its link with the intake of meat from sick animals (N. N. Ginsburg, 1966).

Bacillus anthracis was first discovered under a microscope by the German physician, Pollender, in 1849; however, he was not willing to attribute to it the significance of a pathogen. Independently of him, similar findings were made by the French researchers, Rayer and Davaine (1850). In 1857, F. Brauel', a professor at the Derpt (presently Tartu) Russian Veterinary School, reported on the presence of "contagion" in the blood of people stricken with anthrax and attributed diagnostic significance to it with regard to anthrax (A. I. Metelkin, 1960). However, only Davaine, in 1863, first demonstrated that the bacillary corpuscles consistently found in the blood of experimentally infected animals were the specific pathogens of anthrax. He named them "bacteridia" (bacteridies charbonneuses).

In 1876, Koch was the first to isolate a pure culture of the pathogen of anthrax, and used it with success to infect animals, making a study of the process of development of the bacilli, with formation of spores, in microscopic preparations. In Russia, V. K. Vysokovich, the well-known researcher, obtained the first culture of B. anthracis in 1882 (A. I. Metelkin, 1960).

In 1881, Pasteur proved that it was possible to immunize against anthrax, having developed a vaccine from attenuated B. anthracis for farm animals. Somewhat later, a similar vaccine was developed in Russia by L. S. Tsenkovskiy (1883).

The works of G. N. Mikhin, I. I. Mechnikov, N. F. Gamaleya, V. F. Nagorskiy, N. N. Mari and many others were of outstanding importance to the study of microbiology, pathogenesis, immunology, epizootiology, epidemiology and prevention of anthrax.

In the Soviet Union, a drastic decline of incidence of anthrax, which prevailed on a mass scale in prerevolutionary Russia, was obtained. The system of measures to control anthrax in effect in our country was developed on the basis of the vast experience in preventive work of agencies of the medical-sanitary and veterinary-sanitary inspectorate [surveillance] and the results of research done at medical and veterinary scientific institutions. In this regard, we must mention the names of Soviet researchers, S. N. Vyshelesskiy, N. A. Mikhin, A. A. Vladimirov, N. N. Ginsburg, S. G. Kolesov, I. A. Dobreytser, A. Ya. Zaporozhchenko, E. N. Shlyakhov and many others who made a large contribution to development of the different aspects of the anthrax problem. Extremely important research on the pathogenesis, symptomatology and treatment of anthrax was conducted by the leading clinicians specializing in infectious diseases in our country: A. F. Bilibin, G. P. Rudnev, K. V. Bunin and others. The development of the highly effective STI vaccine by N. N. Ginsburg in 1942 was of enormous significance; it found wide application in the prevention of anthrax among animals and man.

The advances made in the USSR in the control of anthrax made it possible to put to Soviet public health the task of radically reducing the incidence of this infection.

CHARACTERISTICS OF THE PATHOGEN

Morphology: The pathogen of anthrax, Bacillus anthracis, is a relatively large rod, [illegible]-8 and occasionally up to 10 µm in length and 1-1.5 µm in width. It is encountered in two forms, bacillary (vegetative) and sporulated, which has the capacity of forming a capsule that could envelop either individual specimens or several microbial cells together. The capsule is generally quite visible on smears of animal organs; the rods are usually encountered single, in pairs or short chains (Figure 1); on smears of liquid nutrient media they are in the form of long chains (Figure 2). The ends of the rods, at the sites of connection, appear to be chopped off or slightly concave, resembling a bamboo stick with geniculate joints. B. anthracis is wanting in flagella, and for this reason it is immobile, which is its absolute differential character.

Staining: B. anthracis can be well stained with all of the basic aniline dyes. It is Gram-positive. The capsules can be stained with gentian violet (according to Rebiger), Romanovskiy-Giemsa or Ol't (with safranin). Spore staining is discussed below.

Cultural and biochemical properties: The pathogen of anthrax is a facultative aerobe. It grows best on nutrient media with free access of oxygen. Growth is retarded in the absence of oxygen.

Optimum growth temperature is 35-37° in agar and 32-33° in broth (N. N. Ginsburg, 1966). Optimum medium pH is 7.2-7.6, but growth may be observed with fluctuations of pH from 7.0 to 8.0 (6.0 to 8.5 according to G.P. Rudnev).

B. anthracis is not demanding with respect to nutrient conditions, and it can develop in various laboratory media containing amino acids of animal or plant origin--beef-extract agar, broth, Hottinger media, gelatin, milk, plant seed extracts, various carbohydrate media, etc.

On agar nutrient media, B. anthracis usually grows in the form of typical R-form colonies, which are relatively large, dull, with darkened center and fimbriate periphery formed by a plexus of fibers (Figure 3). The latter property motivated naming these colonies "lion's mane," "Medusa's head," etc. Capsules do not form on agar. Old laboratory cultures on agar can form S colonies, which are semitransparent, mucous, with smooth and even margins.

S-form colonies consisting of capsular rods develop on nutrient media containing protein (coagulated horse serum). Cultures that have lost the ability to form capsules yield typical R forms on this medium.

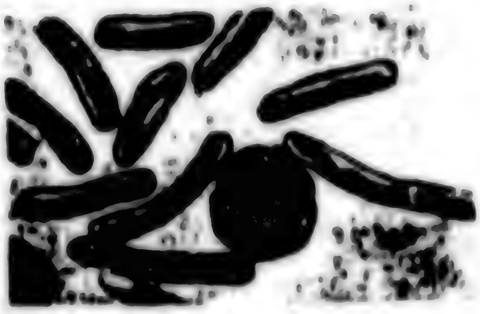

Figure 1.
Encapsulated B. anthracis (replica plated smear of white mouse spleen)

When B. anthracis is cultured in broth, a flocculated sediment appears at the bottom in the form of a cotton ball, while the broth remains clear. Capsules do not form on ordinary broth. When alkalinity of the medium is increased, one may observe clouding in the broth culture (Ye. I. Silant'yev, 1964).

On GKP* medium (see below), there is profuse growth with formation of capsules around the rods. When inoculated by injection into a column of beef-extract gelatin, growth in the form of an inverted fir tree is observed after 3-4 days of culturing at 22°. At the bottom of the formed funnel there is accumulation of white flocculea.

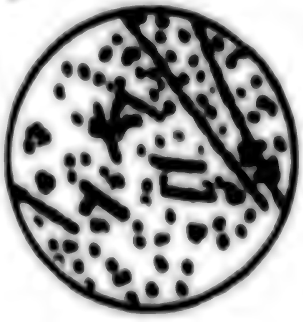

Figure 2.
Smear from culture (spore)

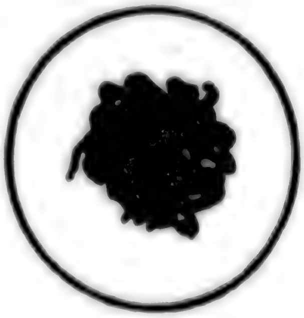

Figure 3.
Young colony at low magnification

When the culture grows on milk, it coagulates within the first 2-3 days, then there is slow liquifaction (peptonization) without formation of acid.

On blood media, hemolysis is usually not observed on the first day; occasionally it does occur at a later time.

Most of the strains of B. anthracis form acid without gas on media containing glucose, fructose, maltose, levulose, trehalose and dextrin. Mild and late acid formation is observed on media with arabinose, rhamnose, mannose, lactose, raffinose, inulin, mannitol, dulcitol [galactitol], sorbitol and inositol; hydrogen sulfide is not always formed; starch is hydrolyzed; no indole is formed (Ye. I. Silant'yev, 1964; N. N. Ginsburg, 1966).

One of the properties of B. anthracis is its high sensitivity to antibiotics (penicillin, streptomycin, biomycin, levomycetin, synthomycin), which affects the cells' capacity to change their form and break down on a medium containing penicillin. At the same time, the antibiotic resistant forms of B. anthracis obtained in the United States are indicative of the possibility of existence of such forms under natural conditions as well (N. N. Ginsburg, 1966).

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*State Control Institute of Medical Biological Products.

Variability: In addition to colonies of B. anthracis of the typical R form, smooth S colonies, dwarf G forms and intermediate pigmented, mucous, etc., forms may be observed. When it changes from the R to the S form, B. anthracis loses the capacity to form chains on smears. Diplobacillary forms are produced, and occasionally the cells are arranged in collections. The cultural and biochemical properties can change; in particular, there is loss of the capacity to dischrage proteolytic enzyme into the culture medium.

Forms that have lost the capacity of capsule formation and, consequently, their virulence, are referable to genetically altered (mutant) forms.

Sporulation: B. anthracis can form spores outside the human or animal body. The following are conditions that cause sporulation: shortage of protein nutrients and moisture, neutral or mildly alkaline medium reactions, absence of calcium. Wheat or mildly alkaline agar prepared on beef broth without addition of peptone is a good nutrient medium for sporulation. Access of free oxygen of air is a mandatory prerequisite. When examining fresh cultures of anthrax in a hanging drop, active sporulation is observed after 24 h only in areas accessible to air along the edges, whereas asporulated forms are found in the middle of the drop. The optimum temperature for sporulation is 30-35°. The sporulating process usually ends after 16 h at temperatures of 30-37°, after 36 h at 24° and it extends to 50 h at 18° (Sobernheim, 1931). There is usually no sporulation at temperatures below 15° and above 42°.

Cultivation of B. anthracis at 42° may lead to loss of the capacity to form spores. Sporogenesis resumes after a series of passages of such cultures at 30-35°. Asporogenic stable variants are obtained with addition to the nutrient medium of potassium bichromate, carbolic or hydrochloric acid.

B. anthracis forms a single centrally located spore, the dimensions of which never exceed the cell's cross section. The spore is oval. Spores are stained by the Peshkov method (methylene blue and neutral red) or Ziehl's fuchsin, similarly to staining of Mycobacterium tuberculosis.

A change in B. anthracis from the sporulated to bacillary form is observed under favorable medium conditions. The optimum temperature is 30-37°, when the vegetation process lasts 8 h; it lasts 16 h at 24° and 70 h at 18° (Sobernheim, 1931). Most authors believe that there is no vegetation at temperatures below 12°.

In the course of changing to the bacillary form, the spores lose their luster, acquire a spherical shape, then become elongated again. They start to resemble young specimens of B. anthracis, the only difference usually being that they have rounder ends; then the bacillus exits from one of the ends in the direction of the longitudinal axis and sheds the spore sheath.

Antigenic structure: Studies of the antigenic structure of B. anthracis revealed several antigenic complexes.

"Somatic" antigen, which contains polysaccharide, was discovered in the body of the bacillus (Ivanovics, 1940, 1958). The polysaccharide of somatic antigen contains equimolecular proportions of N-acetyl glucosamine and galactose, and a negligible amount of O-acetyl and amino acid residue (Smith et al., 1955). The polysaccharide antigen is heat stable, and this is the basis of the Ascoli thermoprecipitation reaction. When boiled, the extract of B. anthracis contains a rather stable polysaccharide fraction, which determines the precipitation reaction through interaction with precipitating serum. Somatic antigen, which is characterized by high serological activity, has no immunogenic or toxigenic properties.

A high molecular protein-like polypeptide of glutamic acid was isolated from the capsular substance of B. anthracis (Tomcsik and Szongott, 1933; Tomcsik, 1933). D-glutamic acid, which is the chief component of capsular antigen, is broken down in animals that are naturally resistant or immunized against anthrax, but it persists in susceptible animals. Like somatic antigen, glutamic acid polypeptid is serologically active, but has no immunogenic or anaphylogenic properties (N. N. Ginsburg, 1966).

Analysis of the antigenic structure of B. anthracis using the gel precipitation reaction revealed four lines of precipitation.

Ye. N. Levina and L. N. Kats (1964) discovered three antigenic complexes in the membrane and capsule using luminescence serological and cytochemical methods: 1) capsular surface antigens, which are apparently peptides that are sensitive to pepsin and, in part, trypsin; 2) intrinsic capsular antigens situated in the main layer of the capsule, which contain protein and polysaccharide substances; they are sensitive to trypsin, chemotrypsin, hyaluronidase and lysozyme; 3) antigens of the cell membrane, which contain both polysaccharide and protein substances, and they are sensitive to lysozyme and trypsin.

B. anthracis produces a special protective antigen (see below) in animals and on certain artificial nutrient media. It is a protein with rather marked immunogenicity.

E. N. Shlyakhov (1957-1962) studied the protein-polysaccharide-nucleic complex contained in B. anthracis, which induces an allergic change in an organism infected with the pathogen of anthrax (see below).

Virulence: Most authors related the virulence of B. anthracis chiefly to the capsular substance of this bacillus. The capsule prevents phagocytosis, protects the bacillus against the bactericidal action of body fluids, and it also causes fixation of the bacillus on body cells, which leads to severe disturbances of metabolic processes in cells, rapid degeneration thereof and death (N. N. Ginsburg, 1966).

Bail (1905) demonstrated that B. anthracis produces, both in vivo and in vitro, a thermostable substance that is stable when heated to 96°, which is localized in the capsule, and he named it aggressin. Aggressin does not have a direct toxic effect, but it can enhance the pathogenic properties of B. anthracis. The loss through evolution of the capacity to form a specific capsule changes the strain of B. anthracis from a highly virulent to avirulent one, so that it can be used for purposes useful to man, to produce vaccine.

Studies of recent years conducted by Keppie, Smith, Harris-Smith (1953), Smith et al. (1954-1955), Harris-Smith et al. (1958), Thorne et al. (1960) revealed that both capsular and acapsular strains produce anthracic toxin. It is destroyed when heated to 60° for 1.5 h; it is also inactivated at pH of less than 5.0 and more than 10.0.

It has been demonstrated that it is lethal to mice, and that it can be neutralized with antiserum obtained from hyperimmunized horses (N. N. Ginsburg, 1966).

Smith et al. (1954-1955) was able to isolate three components of the toxin by ultracentrifuging and filtration thereof, and their combined effect induced edema of the rabit integument and was fatal to mice, whereas none of these components separately had such properties. Strange and Thorne (1958) isolated two components from anthracic toxin of a factor that was retained in the filter, each of which had no toxinogenic or immunogenic properties, as well as a factor that passed through the filter and had immunogenic properties, without toxicity, the "protective antigen." The combination of the factor that passes through the filter and second component of the factor that is retained on the filter has toxigenic and immunogenic effects (quoted from N. N. Ginsburg, 1966).

Resistance: The vegetative forms of B. anthracis are relatively unstable: they perish within 40 min at 55°, 15 min at 60° and immediately at the boiling point. Low temperatures are less devastating for the vegetative forms of B. anthracis: they survive for 24 days at -10°, 12 days at -24°, and even at -180° live bacilli are found after 3-15 h. Direct sunlight kills them within a few hours. Various disinfectants (5% carbolic acid, 1% corrosive sublimate, 5% calcinated lime, formalin, hydrogen peroxide)kill the vegetative forms within a few minutes. The bacilli remain viable for several months in water. The vegetative forms of B. anthracis perish within 2-4 days in cadavers that have not been autopsied (7 days in marrow) as a result of the influence of putrefactive flora and absence of oxygen.

The spores of B. anthracis are notable for their extremely high resistance. They survive for several years without oxygen and when dried. The spores are still capable of vegetating after boiling for 5 min. They perish within 12-15 when exposed to flowing steam at 100° and within 5-10 min at 110°. Dry heat kills the spores within 3 h at a temperature of 140° and within 1 h at 150°. A 1% formalin solution and 10% solution of caustic soda kill the spores in 2 h.

Some authors report that the degree of resistance of spores of different strains may depend on the conditions under which sporulation occurred. Thus, S. N. Vyshelesskiy and F. A. Terent'yev (1954) indicate that the spores formed at temperatures of 18-20° are more stable than at 35-38°.

Anthracic bacteriophage: The first information about anthracic bacteriophage is referable to 1898, when N. F. Gamaleya first discovered the phenomenon of lysis in a bacterial suspension of B. anthracis.

In 1929, F. N. Rozgon isolated a specific phage of B. anthracis from Tsenkovskiy's vaccine strain. In subsequent years, Cowles (1931), F. A. Terent'yev (1937) and A. P. Zotov (1940) isolated phages active for B. anthracis from liquid sewage, animal excreta and old laboratory cultures. In 1951, McCloy isolated bacteriophage W from an atypical strain of B. cereus, which induced lysis of all (171) tested strains of B. anthracis and only isolated cultures (2 out of 56) of B. cereus. Subsequent studies of various strains of anthracic bacteriophage were conducted by Brown and Cherry (1955), Ivanovics and Lantos (1958) and others (quoted from N. N. Ginsburg, 1966).

In 1961, Ye. V. Gruz isolated the highly specific anthracic bacteriophage BA-9 from the soil of Kishinev. In 1962, anthracic phage was isolated by A. Ya. Meshcheryakov. In 1964, V. S. Larina and L. S. Petrova reported that they isolated a specific phage which they arbitrarily called Saratov. I. M. Zemtsova (1965), Ye. N. Levina and V. R. Arkhipova (1967) studied the biological and antigenic properties of various anthracic bacteriophages.

At the present time anthracic bacteriophages have begun to be used extensively for identification of B. anthracis.

GEOGRAPHIC DISTRIBUTION

Analysis of the data of the International Epizootiological Service (FAO [Food and Agriculture Organization of WHO]) pertaining to incidence of anthrax among animals in different countries of the world shows that the largest number of outbreaks in Europe is recorded in countries located in the southern part of this continent. In 1959-1964, there were 7871 outbreaks of anthrax among domestic animals in Greece, 1837 in Italy, 1215 in Spain, 1144 in Bulgaria and 1084 in Yugoslavia. At the same time, the number of recorded outbreaks in north European countries was relatively low. Thus, in 1959-1964, there were 11 outbreaks of anthrax in Norway, 16 in Sweden, 22 in Finland, etc.

Among the Asian countries, the most frequent outbreaks of anthrax in animals were encountered in India, Iraq, Iran, Turkey, Burma, eastern Pakistan. Between 1959 and 1964, there were 5137 outbreaks among animals in Iran, 4748 in Turkey and 4238 in India. The incidence was also high in some other countries. For example, there were 4140 cases of anthrax among animals in Syria in 1959-1964.

On the African continent, the largest number of outbreaks is observed in the South African Republic, Sudan, Ghana and Morocco. Between 1959 and 1964, there were 573 outbreaks of anthrax among animals in the South African Republic and 215 in Morocco. A total of 817 outbreaks were recorded in Sudan in 1960-1964 and 163 in Ghana in 1961-1964.

The data pertaining to animal morbidity in American countries are rather incomplete. Of these countries, we can mention Brazil, where 248 outbreaks of anthrax among farm animals were observed between 1959 and 1964. The FAO data also indicate that 515 animals were stricken with anthrax in Venezuela in 1959-1964, 7276 in Chile in 1956-1960 and 129 in the latter country in 1963. In Argentina, 3794 animals were stricken in 1956-1958 and there were 439 outbreaks in 1959-1962.

According to the data of WHO, there were 60,618 cases of anthrax among the inhabitants of the world over a 10-year period (1955-1964) (Table 4).

The data listed in Table 4 indicate that the number of recorded cases of anthrax among people is declining. In the last 5 years of the period analyzed (1960-1964), there were 1.9 times fewer cases than in the first 5 years (1955-1959). This trend is seen on all continents: 2.1 times fewer cases in European countries in the last 5 years, 1.1 times fewer in American countries, 1.3 times fewer in Asian countries and 1.7 times fewer in Africa.

Table 4. Incidence of anthrax among the population of different continents in 1955-1964 (according to WHO data)

Years / Continent	1955	1956	1957	1958	1959	1960	1961	1962	1963	1964	1955–1959	1960–1964
Europe	3 962/15	3 611/18	2 544/15	2 775/14	1 655/14	1 680/15	1 484/12	1 480/12	1 159/11	1205/10	14 527	7017
Asia	1 736/12	2 116/12	1 776/13	1 525/10	1 567/15	1 129/7	1 672/9	1 302/8	[illegible]/4	2420/9	8 720	[illegible]
Africa	1 771/16	2 207/12	1 512/14	1 939/15	1 752/20	2 070/18	1 187/19	1 094/11	147/6	551/7	9 182	[illegible]
America	1 232/14	875/13	1 108/13	907/10	681/11	1 076/10	724/12	783/6	945/13	865/12	4 803	4 393
Australia and Oceania	...	13/2	...	4/1	...	4/1	14/9	5/2	...	...	17	22
Entire world	8 703/57	8 622/57	6 940/55	7 129/50	5 755/59	5 958/51	5080/47	4751/40	2477/34	4950/41	37 219	22 309

Note: Number of cases in numerator and number of countries, in which cases were recorded in denominator; ... indicates that no information is available.

In 1955-1959, 38.5% of all cases among people were noted in European countries; 23.6 and 24.9% were recorded in Asia and Africa, respectively, and 13% in the Americas. In the last 5 years this proportion changed somewhat: only one-third of all cases of anthrax were recorded in Europe (30.0%), with concurrent increase in incidence of the disease in America (18.5%). The figures were 23.0% for Africa and 28.5% for Asia. There are only isolated cases of anthrax among the people of Oceania.

In the Soviet Union, annual mass scale immunization of cattle is lowering the incidence of anthrax. According to the data of S. G. Kolesov (1964), due to the increased scope of inoculation of farm animals by 3.1 times, the incidence of anthrax decreased by 6.2 times while the number of deaths decreased by 7.8 times. At the same time the incidence of anthrax among people is dropping drastically. According to the data of N. N. Ginsburg and B. L. Cherkasskiy (1965), the incidence decreased to 1/32d in 1965, as compared to 1913, to 2/7ths as compared to 1950 and to 10/23ds as compared to 1955.

At the present time, anthrax is observed mostly in the northern Caucasus, Transcaucasia, Kazakhstan and Central Asia.

A. Ya. Zaporozhchenko (1964), who analyzed 2400 cases of anthrax recorded in the USSR between 1920 and 1959, determined that 26.64% of the cases were referable to Central Asian republics, 36.66% to northern Caucasus and Transcaucasia, 19.6% to Povolzh'ye (Central and Lower Volga), 10.80% to western Siberia, 1.25% to eastern Siberia, 5.10% to southeastern Ukraine and 0.41 to southwestern Ukraine.

EPIZOOTIOLOGY

Domestic animals--sheep, goats, cows, buffaloes, horses, donkeys, deer and camels--are considered to be the most susceptible to anthrax infection. Pigs are less susceptible.

A large number of reports concerning anthrax among wild animals has been accumulated in the literature in the last 80 years. Spontaneous infection and deaths of animals due to anthrax were demonstrated in western Europe, in the USSR (tundra, central belt of its European part and western Siberia, Crimea, Azerbaijan, Kazakhstan, Kirgizia, Tadzhikistan), Canada, United States, South African Republic and Liberia.

Among the wild animals stricken with anthrax in their natural habitat, there is prevalence of Ungulata, especially representatives of the Cervidae and Bovidae families. Death of moose due to this infection was observed in the Baltic region in 1752, 1865 and 1885, and in this century in Tul'skaya Oblast, Mordovskaya ASSR and other areas.

Major epizootics of anthrax were recorded in 1962-1963 among bisons in the Slave River valley (Canada), when 562 animals perished (Cousineau, McClenaghan, 1965), and among deer in Arkansas (United States) in 1964, when 70-90% of the animals died. Anthrax has also been reported among mountain sheep, roe deer, aurochs and wild boars.

An enormous epizootic of anthrax was observed in Kruger Park, in the South African Republic (Piennaar, 1961), where 1054 carcasses of various animals that died of anthrax, including 771 kudu antelope, were found between June and October 1960. In addition to antelopes of many species, buffaloes, warthogs, zebras and giraffes died there.

All these facts indicate that representatives of different species of wild Ungulata are just as susceptible and sensitive to anthrax as domesticated species. This should have been expected, in view of the significant similarity of domesticated and wild animal species.

In recent years, an increasing number of reports is appearing concerning the isolation of B. anthracis from different rodent species. Thus, we know from the report of I. G. Galuzo (1954) that cultures of B. anthracis were isolated from the little souslik in Kazakhstan. V. M. Tumanskiy (1958) reported that B. anthracis was isolated in the natural habitat of sousliks by Anokhina and later Shumkova. Ye. Ye. Punskiy and F. S. Tsibulevskaya (1958) isolated the pathogen of anthrax from the great gerbil in Turkmenia. S. N. Rybin, V. F. Zaglyadina and I. P. Gordiyenko (1964) described one instance of isolating B. anthracis from a long-tailed marmot in

Kirgizia, [illegible] Nadzhafov (1965) isolated it from red-tailed Libyan jirds in Azerbaijan, while M. A. Musayev, F. A. Abushev and S. Kh. Yuditskaya (1966) isolated it from common field mice. Unlike wild Ungulata, B. anthracis was not isolated from dead rodents, but from clinically healthy animals. These facts are indicative of the unquestionable possibility of anthrax infection among a number of rodent species in their natural habitat.

As for carnivorous mammals, spontaneous anthrax infection has been demonstrated in only a few species. They are mainly predators that hunt for Ungulata and consume smaller warm-blooded animals: lions, leopards, cheetahs, genets, honey-badgers (Piennaar, 1961) and wolves (I. I. Kryuchkov, 1953).

We also know of numerous instances of infection of carnivorous mammals in zoos and fur farms after eating flesh from wild or domestic Ungulata that died or were suffering from anthrax. Such cases have been described for foxes (L. A. Rozen'yer, 1948; Yu. A. Myasnikov, Z. A. Levacheva, 1963), raccoons, bears, leopards (L. A. Rozen'yer, 1948), Arctic foxes, sable (A. A. Sludskiy, 1954) and others.

There are known cases of fatal anthrax infection among predatory birds. In particular, Stip (quoted from L. A. Rozen'yer, 1948) reported on the death of 50% of the eagles, falcons and owls at the Moravian Zoo, which were fed meat from animals that had died of anthrax.

There are more numerous reports of the fact that carnivorous animals and birds, infected with anthrax as a result of eating sick animals or those that died of anthrax, which did not get sick but were capable of eliminating B. anthracis spores in feces and of infecting soil during their hunting expeditions in areas that were sometimes quite remote from the sites of initial infection. Such findings have been made with regard to foxes, jackals, coyotes, American black vultures, sparrowhawks, kites, falcons and others.

There has been experimental demonstration of high sensitivity to anthrax of white mice, guinea pigs, rabbits and monkeys.

The alimentary route is the main route of infection with anthrax of animals in the wild. The mucosa of the digestive tract is the point of entry of the infection. The animals are usually infected by grazing on herbage contaminated with anthracic spores.

For this reason, the soil, which not only serves as the site of B. anthracis in the environment but, in the opinion of a number of authors, is the place where there is accumulation of the pathogen under specific physicochemical and biological conditions as a result of repeated vegetations, is of enormous importance to the epizootiology of anthrax (A. A. Vladimirov, 1936; K. A. Mirotvorskiy, 1940; N. A. Mikhin, 1942, and others).

Sick animals eliminate B. anthracis in urine, excrements and saliva, which get into pasture soil. During their last minutes of life and first hours after death due to anthrax, the animals often discharge a bloody liquid from the mouth, nostrils and rectum, and this liquid contains B. anthracis that subsequently forms spores in the soil. The soil may also be contaminated as a result of being saturated with blood after slaughtering anthrax-stricken animals in the pasture, skinning animals, cutting up the carcasses of dead animals, etc. Scattering of parts of carcasses of animals that died of anthrax by carnivorous animals and predatory birds, use of infected bone meal as fertilizer, hauling silt from infected bodies of water to the fields, etc., may cause contamination of soil with anthracic spores.

The exceptional resistance of anthracic spores in the environment results in the fact that, in a number of instances, contaminated soil presents a potential hazard to herbivorous animals for dozens of years. Mass scale vaccination of cattle lowers drastically the epizootiological hazard of such soil, but does not eliminate it completely, since even under these conditions some animals, which have not been inoculated for some reason or other, are exposed to the risk of infection and often are stricken by anthrax. Flooded rivers and rain water that erode soil, plowing of areas where animal carcasses are buried, various types of agricultural work, the scope of which has increased significantly in our country in recent years in view of deployment of reclamation measures, construction, development of new land, etc., may serve as factors causing movement of spores f ~ deep soil layers, infection of surface layers in pastures and, consequently, onset of anthrax among cattle. The layers of soil containing spores of B. anthracis often become exposed, cast on the surface and scattered in the environment.

M. K. Timofeyev, V. N. Karelina and P. P. Kolushev (1962) described an outbreak of anthrax among animals along the Arzamas-Gor'kiy cattle-driving route.

Anthrax spread over many flocks as a result of contact of cattle with the infected soil of trenches that were dug to install gas lines. Many animals died and several people were stricken. N. M. Noskov (1967) reports that, in 1964, an epizootic outbreak of anthrax occurred in one of the villages of Kirovskaya Oblast 76 years after the last case of the sickness among animals. Appearance of the disease was preceded by ejection and scatter of soil on pastures as a result of seismic work related to well-drilling and subsequent explosions. Buchlmann (1962) described a case observed in Austria, when spores of B. anthracis were carried to the soil surface as a result of washing out humus over the meadow by sewage flooding, which caused the disease among cattle that grazed there 70 years after the last cases of anthrax recorded in that locality.

The name of "damned fields" was given to the soil of fields, meadows, grazing land infected with B. anthracis and presenting a danger to herbivorous animals for many years.

Endemic anthrax sites in soil have been found in many countries. In the United States, the stricken areas are in southeastern South Dakota, northeastern Nebraska, a strip of land along the coast of Texas ["Bay"], the delta region of the Mississippi, including parts of the states of Arkansas, Mississippi and Louisiana, the valleys of the San Joaquin and Sacramento rivers in California (Stein, 1955). In Bulgaria, more than 1200 settlements situated in the valleys of the Danube, Maritsa, Tundzha, Yantra and other rivers are surrounded by "damned fields" (N. Dimitrov and Kh. Panchev, 1962). In France, the most stricken areas are Beauce, which is long known as the "classical country of anthrax," as well as Brie, Bourgogne, Lorraine, Languedoc, Provence and others (Nocar and Leclenche, 1897). In Italy, one of the worst areas with respect to anthrax is the region of Apulia, mainly the province of Foggia (Taselli, 1963). There are numerous endemic soil sites of anthrax in other countries as well.

However, not all types of soil are equally beneficial for the survival of the pathogen. In some cases, it not only remains viable, but conditions are favorable for its vegetation. In other soils, the conditions cause gradual loss of virulence of B. anthracis, or else inhibit its vital functions, by virtue of the specifics of physicochemical and biological properties.

Many authors have concluded, as a result of analyzing territorial distribution of areas that are consistently adverse for anthrax, that it is expressly the nature of the soil where they are located that is one of the chief factors determining the territorial location of these areas. For example, it is considered proven that chernozem soil is the most beneficial for preservation of the activity of endemic soil sites.

A. V. Koronnyy (1958), who studied the territorial distribution of anthrax sites with due consideration of their epizootiological activity in one of the regions near Moscow over a 60-year period, observed that there were 6 times more outbreaks of anthrax among cattles in chernozem areas than podzol sandy loam.

According to the data of A. S. Korotich and K. S. Danilova (1960), 50.7% of the sites permanently stricken by anthrax in the Ukraine are located on chernozem soil, 31.6% on chernozem with podzolized soil, 9.9% on podzolized soil, 5% on soddy podzolic and brown soil, and only 2.8% on bog and soddy podzolic soil.

According to the data of V. Ya. Sklyarov (1964), 80% of the 537 sites with an anthrax problem found in northern Caucasus are situated on chernozem soil.

In Kokchetavskaya and Severo-Kazakhstanskaya oblasts of Kazakhstan, where the area occupied by chernozem (ordinary and southern) constitutes 33.2-35.6%, 4.4-10.3% of the populated centers, respectively, have an anthrax problem, whereas in Pavlodarskaya and Kustanayskaya oblasts, where chernozem covers only 3.6-17.8% of the area, only 2-2.8% of the settlements are a permanent problem with respect to anthrax (B. L. Cherkasskiy, 1967).

Several researchers report that the regions where the most active and permanent sites of anthrax are located are usually characterized by high humidity or swamps, and they are situated in the floodplans of rivers or near brooks. During periods of high water [floods], B. anthracis spores are washed out of the soil to the surface, and sometimes they are carried away over great distances.

The water factor may also be significant in the spread of B. anthracis in the environment when the soil and water supply sources are contaminated with infected liquid waste from tanneries, wool-washing plants and other industrial enterprises that process raw material of animal origin.

The problem of eradicating anthrax is directly related to the search for effective methods of detecting and treating infection sites in soil. This problem unites the interests of epidemiologists and epizootiologists, and it requires their joint efforts to be resolved.

One of the important factors in transmission of anthrax to animals is synthetic feed. It consists of many components of both plant and animal origin, including bone, meat and blood meal. Cases of anthrax among animals due to infected meat meal contained in synthetic feed were reported in Sweden and cases related to blood meal were noted in the United States. Transmission of infection by imported bone meal has been reported in the United States, FRG and Great Britain (Kauker, 1965).

Studies of outbreaks of anthrax among domestic animals in the midwest region of the United States in 1952 revealed that virtually all of them were related to feed of poor quality. For example, B. anthracis was isolated in Ohio from imported bone meal fed to pigs (E. N. Shlyakhov, 1957). These outbreaks were characterized by extensive spread of the disease, occurrence in the wintertime in areas that had previously not been stricken, and absence of recurrence among pigs or other animals kept together.

Piening (1958) believes that bone meal infected with anthrax, produced in FRG from bones shipped from many countries of the world was the cause of the major outbreak of anthrax among dairy cows in farms of all parts of Schleswig-Holstein (FRG) in January to April 1958. This was corroborated by the fact that this feed was used only at farms in Schleswig-Holstein. In addition, in the course of the outbreak only adult animals fed bone meal were stricken, whereas young livestock that was not given this feed did not get sick.

Equipment related to animal upkeep, for example, harnesses removed from animals that died of anthrax and then put on healthy cattle, brooms contaminated with anthracic spores used to clean the troughs, etc., may also serve as factors of transmission of anthrax infection.

There is relatively rare infection of animals through the broken skin, nasal mucosa or conjunctiva. There are also only rare cases of intrauterine infection. Under artificial conditions, it is easy to transmit the infection to animals via the respiratory route.

There may be transmissive transmission of B. anthracis. On this basis, V. N. Beklemishev (1956) classifies anthrax as a facultative-transmissive infection, with which the "transmission by arthropods under specific conditions could cause outbreaks to persist and spread, but the pathogen can also spread without a vector for an indefinite time."

Blood-sucking arthropods implement the transmissive mechanism of further spread of infection after sucking the blood of sick animals that contains B. anthracis during the period of bacteriemia. As they pursue animals, blood-sucking arthropods, particularly horseflies, can spread infection over dozens of kilometers, alternating active flight with passive transportation on the animals. In addition, horseflies can be infected directly by contaminated water reservoirs where they slake their thirst, as well as soil that they lick with their proboscis. According to the observations of P. P. Lelep (1936), horseflies can infect soil, grass and small bodies of water with B. anthracis, dispersing the excesses of consumed blood in the process of defecation.

Gadflies (genera Tabanus L., Chrysops Mg., Chrysozona Mg.), flies (Muscidae, Calliphoridae, Sarcophagidae, Hippoboscidae families), mosquitoes (Culicidae family) and gnats (Similiidae family) transmit B. anthracis.

The involvement of ticks in transmission of B. anthracis is still debatable, although some authors mention this possibility with reference to various species of Argas (Ornithodorus moubata, Argas persicus), Ixodes (I. ricinus, Boophilus decoloratus) and gamasid mites (Hl. glasgowi).

The tayga and forest belt of our country is characterized by a particular profusion of horseflies and other blood-sucking Diptera. Horseflies and other blood-sucking insects were the most important factor in transmission of anthrax infection during the mass scale epizootics among horses and cattle, which occurred repeatedly in the tayga and forest regions before the revolution (V. Nagorskiy, 1904). N. G. Olsuf'yev and P. P. Lelep (1935) also stress the significance of horseflies in the spread of anthrax in the landforms of the forest zone, as well as floodplains and flood regions of large rivers in the steppe and forest-steppe zones. In the central and southern zone of the USSR, Stomoxys and Lyperosia stable flies, as well as various blood-sucking insects play some epizootiological role. In the tundra region, mosquitoes, which are particularly profuse there, play a large role in the spread of B. anthracis (N. G. Olsuf'yev and P. P. Lelep, 1935).

The seasonal nature of anthrax is attributable to the distinctions of infection-transmitting factors prevailing in different countries. Most cases occur in January-March in Denmark, FRG and GDR; in February and March in Switzerland, Argentina; in May in Portugal and Czechoslovakia;in July-September in the Soviet Union, Hungary, Romania, Spain, Turkey and India; in August-September in Bulgaria, France, Poland, Iran, Tunis, Liberia; September-October in Italy, Yugoslavia, Iraq, Morocco; October-December in Belgium, Great Britain, the Netherlands and Australia.

If we consider that, for Australia and Argentina, February and December are summer months, it becomes apparent that seasonal rises in morbidity are inherent in the warm part of the year in most of the countries listed. In view of the fact that cattle are in pastures at this time of the year, most cases of anthrax in the above-mentioned countries are apparently related to infected soil sites.

The maximum incidence of anthrax among cattle in the summer months is attributable to the fact that, when there is a drought characterized by a profusion of dust, the animals swallow many spores along with soil particles. In hot and dry summers, when the grass burns out in the pastures, the cattle are often driven to drying swamps, ditches, abandoned cattle-burying grounds and other areas where the carcasses of anthrax-stricken animals had previously been dumped. The dry stems of plants and rootstock consumed during periods when the pastures are bare injure the mucosa of the mouth and esophagus, cause development of infection in the animals. Migration [flight] of horseflies and other blood-sucking arthropods involved in the spread of infection among animals also determines the formation of the summer season of anthrax.

In countries where anthrax occurs in the winter (Denmark, FRG, GDR, Switzerland, Belgium, Netherlands, Great Britain), most cases of the disease are caused by B. anthracis spores brought in with imported artificial feed.

EPIDEMIOLOGY

The sources of anthrax for man are sick animals or animals that died of this infection. As a rule, infection from man to man is not observed, due to the absence of the mechanisms of transmission of infection inherent in anthrax in the intercourse among humans.

The pathogens are transmitted from animals to man over various routes, with the participation of diverse factors. Transmission may occur through the contact route by means of direct contact with the source of infection or transmission factors, as well as alimentary, transmissive and air-borne routes. The contact mechanism of transmission of B. anthracis prevails over the others (Figure 4).

The clinical form of human infection is determined by the mechanism of transmission of the pathogen. If the pathogen penetrates through the broken skin, in the case of contact and transmissive mechanisms of transmission, the cutaneous form of anthrax occurs. Swallowing the pathogen could cause the intestinal form, while inhalation could cause the pulmonary form of infection. By virtue of prevalence of mechanisms of transmission that cause the pathogen to penetrate the integument, the cutaneous form of anthrax is observed many times more often than others. About 99% of all cases of this disease are referable to this form. This prompted L. V. Gromashevskiy to classify anthrax in the group of integumental infections, on the basis of the chief primary localization of the pathogen.

Human infection may occur while caring for sick animals, forced slaughtering thereof, skinning them and cutting up carcasses. After contact with a sick animal or one that has died of anthrax, the pathogen gets on the integument and, if it is broken (bruises, cuts and other minor trauma) infection will occur. The cutaneous form of anthrax results from this route of infection.

The cutaneous form of anthrax may also be a consequence of the transmissive mechanism of man's infection from a sick animal or one that died of anthrax through a blood-sucking arthropod. This route of transmission of anthrax to man has been described by many researchers. According to official records, 0.2% of human cases were the result of transmissive transmission of the pathogen in the Soviet Union, in 1966.

G. P. Rudnev makes a distinction between three types of anthrax, depending on their origin: 1) occupational-agricultural; 2) occupational-industrial and 3) domestic [in the course of everyday life].

The type of morbidity related to infection from sick animals are animals that died of anthrax is also called the livestock type (L. V. Gromashevskiy and G. N. Vayndrakh,

1947) or agricultural (G. P. Rudnev, 1959). Depending on whether the victims in such cases are employed in public livestock raising (shepherds, employees on livestock ranches, veterinary and zootechnical specialists) or are owners of private cattle, or individuals who came in contact with such cattle by chance, i.e., depending on whether the contact with sick cattle was domestic of occupational, a distinction is made between livestock-domestic or livestock-occupational (occupational-agricultural) types of anthrax.

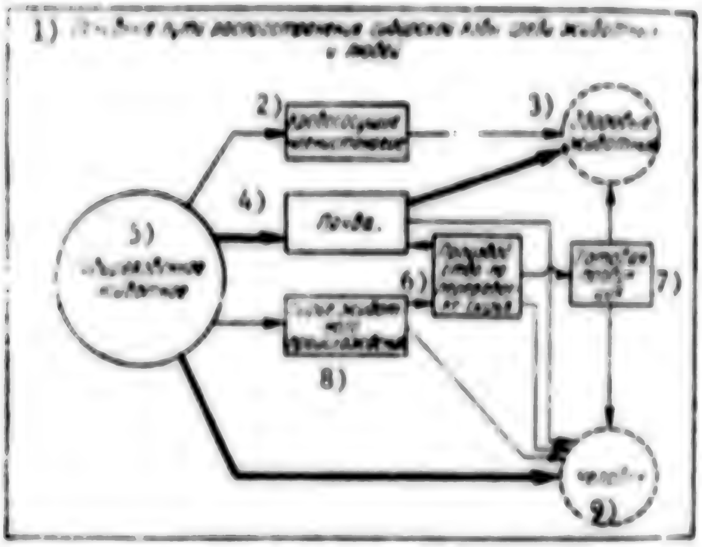


Figure 4.
Main routes of spread of anthrax among animals and man (after B. L. Cherkasskiy)
Key:

1) main routes of spread of anthrax among animals and man
2) blood-sucking arthropods
3) healthy animals
4) soil
5) animal stricken with anthrax
6) enterprise that processes raw material
7) end product
8) raw material of animal origin
9) man

In many countries, contact with sick animals is one of the main mechanisms of transmission of infection to man. For example, in Italy, chiefly shepherds and farm workers are stricken with anthrax (Taselli, 1962). In Australia, two-thirds of the anthrax cases in 1951-1960 had some connection with slaughtering animals (Puntigam, 1963), etc. In the Soviet Union, about 90% of the anthrax cases are recorded in rural areas. This is attributable to the fact that in almost three-quarters of the cases the infection occurs as a result of direct contact with sick animals or animals that died of anthrax, mainly those owned by the private sector and not submitted to preventive inoculations.

Anthrax may also develop as the result of man's direct contact with soil infected with B. anthracis. Thus, 8.7% of the cases between 1947 and 1963 in Volgogradskaya Oblast occurred from infection through the soil (A. Suvorova, 1964). In 1961, soil was the transmission factor in 9.4% of the cases in Azerbaijan SSR and 9% of the cases in Georgian SSR (N. I. Makarov et al., 1963). According to the records, in 1966, 4.7% of the anthrax cases in the Soviet Union were due to infection through soil, such cases occurring in 15% in Azerbaijan SSR, 10.8% in the Ukraine, 6.2% in Kazakh SSR, etc. In spite of the relatively low share of cases due to man's direct infection through soil, the very fact that they occur indicates once more the extremely great importance of the infected soil sites in the problem of anthrax as a whole.

Meat, pelts, wool, bristles, hair and other livestock products recovered from animals stricken with anthrax are important factors in transmission of B. anthracis to man.

Consumption of meat from animals stricken with anthrax without adequate heat treatment may cause the intestinal form of anthrax in some cases. One such epidemic was described by V. Barykin, G. Vygodchikov and Ye. Sazhina (1929). In 1927, at a sausage workshop referable to the cottage industry in Yaroslavl', a small batch of sausage was produced from the meat of a cow that had to be destroyed. As a result of consuming this sausage, 23 people were stricken with the intestinal form of anthrax. This epidemic of intestinal anthrax was the largest of those described in the literature.

In the Soviet Union, only 6 outbreaks of intestinal anthrax were described for the period between 1923 and 1940 (64 cases). Alimentary infection was related chiefly with intake of cooked sausage made form the meat of anthrax-stricken animals. The technology of production does not provide an adequate temperature in such sausages to decontaminate anthracic spores. At the same time, the limited extent of the observed outbreaks of intestinal anthrax indicates, apparently, that additional conditions are required for them to occur, in particular some lesion to the mucosa of the digestive tract, etc.

These factors are also involved in the very low incidence of intestinal anthrax due to intake of milk from stricken animals or infected water.

The products of livestock derived from animals stricken with anthrax serve much more often as the cause of industrial-occupational cases of anthrax among individuals who collect, store, transport and process raw material of animal origin in the tanning, sheepskin coat, wool, bristle brush, shoe, glue-making, heat-recovery and a few other sectors of industry. In these industries, processes related to raw material prior to its processing and the early stages of processing are particularly hazardous.

In the industrial shops of tanneries, employees involved in the first stage of hide processing most often contracted anthrax, i.e., those in the soaking and ashing department, whereas the subsequent industrial processes (tanning, greasing, drying, mechanical cutting, dyeing) present much less danger of infection, since the hides at these stages are already free of wool and scrapings, they have been washed and treated with alkali, acids and tanning agents.

At wool factories, processes dealing with untreated raw material and the early stages of processing are also the most hazardous. Anthrax was observed most often among loading hands, warehouse personnel, sorters, workers in the scutching and instrument shops, whereas the disease was encountered much less often in the weaving and spinning mills, to which the wool arrives after being dyed and boiled, washed in hot water with soda and dried (N. A. Yablokov, 1950).

At the present time, anthrax strikes primarily the representatives of some occupational groups in many countries: transport and port workers of the tanning, textile and other enterprises that process raw material of animal origin, fur and pelt traders, etc.

For example, in the United States, anthrax has been recorded in recent years mainly in Pennsylvania, New York, New Jersey, Massachusetts, New Hampshire, Delaware and Connecticut, where most of the tanning, wool and hair-processing industries are concentrated. The spread of industrial anthrax is due primarily to processing of wool and hair imported from countries of western Asia and some countries of southern Europe (Greece, Italy, Portugal, Spain, Malta and others) (E. N. Shlyakhov, 1957; O. V. Baroyan, 1962, 1967). All of the cases of anthrax among people that were recorded in the United States in 1961 and 1963 were industrial-occupational in nature.

Cases of pulmonary anthrax are linked with the use of infected raw material of animal origin. Anthracic spores remain viable, even when the raw material is dried. In view of the considerable amount of dust in industrial buildings, due to the technology of processing such raw material, spores can be scattered with the flow of air and invade the human body via the respiratory route.

Such factors caused the form of pulmonary anthrax, which was widespread in the past, that was known by the name of "rag-sorter's disease." This occupational form of the disease was observed in ragpickers who collected rags from dumps that were contaminated by manure from sick animals. At the present time, rag-sorter's disease is no longer encountered, since the manure and carcasses of anthrax-stricken animals do not reach dumps because of the extreme rarity of anthrax among animals in cities, as well as intensification of veterinary surveillance (L. V. Gromashevskiy and G. M. Vayndrakh, 1947).

At the present time, pulmonary anthrax is encountered in the form of rare cases of occupational infection in some sectors of industry. For example, in Germany, 7.8% of all cases of anthrax recorded between 1910 and 1938 were due to inhalation of dust containing anthracic spores in areas where wool and pelts were processed (Puntigam, 1963). In the United States, there was an outbreak of anthrax in November 1957, in New Hampshire, at a factory that processed goat's wool. In the course of this outbreak 9 people were stricken, 5 of whom had the pulmonary form of anthrax and 4, the cutaneous (Brachman, Plotkin, Bumford, Atchison, 1960).

The data of Carr and Rew (1957) are indicative of the epidemic hazard of raw material of animal origin; they demonstrated B. anthracis in smears and nasopharyngeal washings from 7 women workers after they had worked for 1.5-4 h with unwashed wool at enterprises that processed goat's wool.

The occupational-industrial cases of anthrax in several foreign countries are due chiefly to the lack of regularly scheduled immunoprophylaxis of this infection among the public. For example, at enterprises of the United States where imported raw material of animal origin is processed, only the administrative personnel is immunized. A small group of people whose work involves a particular risk of infection is inoculated at the request of the Center for Communicable Disease Control under the Health Department ("Report of the U. S. Epidemiological Center," 1964).

In prerevolutionary Russian, industrial-occupational cases of anthrax constituted almost one-third of all cases recorded in the nation (N. A. Mikhin, 1942). According to the data of Birger, Korobitsina and Nabokov (quoted from V. A. Bashenin, 1955), 27 (57.4%) out of 47 cases of anthrax in Moscow, in 1926, were related to industry: 13 cases occurred in connection with wool processing, 9 in the tanning and fur industry, 5 in the hair and bristle processing industry.

At the present time, as a result of implementation of a system of protective measures, such cases are recorded relatively seldom (3.6%) in the Soviet Union.

The low incidence of industrial-occupational anthrax is attributable as well to the effectiveness of the sanitary rules established in the USSR for procurement, storage, transportation and processing of raw material of animal origin, which protect individuals working in the processing industry against anthrax infection, along with the broad coverage of appropriate groups with regularly scheduled inoculation. Also important is the fact that procurement of animal raw material is permitted exclusively in regions that have no animals with communicable diseases. Each batch of raw material delivered to an enterprise can be accepted to a warehouse or processing only with the permission of the veterinary sanitary inspectorate that services the enterprise. Disinfection of contaminated raw material or destruction thereof is also performed under the supervision of veterinary personnel. Employees at enterprises engaged in processing animal raw material are furnished with work outfits [coveralls], personal hygiene supplies, etc.

In view of the regularly scheduled implementation of anthrax-control measures, and especially in view of the system of quarantine measures, imported raw material of animal origin has no appreciable significance as a factor in transmission of anthrax infection in the Soviet Union. However, the frequent cases of anthracic spore infection of pelts and wool in raw material imported from a number of Asian, African and Latin American countries are indicative of the constant hazard of this factor, and they require the extreme vigilance of quarantine surveillance.

Not infrequently, occurrence of cutaneous anthrax is related to the household use of products made of infected animal raw material: new sheepskin coats, hats, felt boots, shaving brushes, etc. In such cases, injury to the integument by the hard edges of clothing, scarification of the skin while shaving, etc., cause infection. Such cases are usually isolated, since the animal raw material processing usually kills the pathogen of anthrax. However, in the case of centralized supply for large groups, there may be outbreaks of anthrax due to items made of raw material of animal origin.

Such an outbreak was described in the Russian army during the Russian-Japanese war in Manchuria in 1904, when 956 cases of cutaneous anthrax occured within a few months as a result of wearing sheepskin coats, fur hats, woolen gloves and other clothing made from contaminated raw material (V. A. Bashenin, 1955).

The cases of anthrax caused by infected shaving brushes are well-known. Thus, during World War I, 46 cases of anthrax were recorded in the English army, which were due to these factors of transmission of infection. In the American army, there were 20 such cases in 1917. A study revealed that 75% of the shaving brushes were made of goat hair obtained from Japan, which was infected with anthracic spores.

There is a graphic case of anthrax in a worker, in the state of Ohio (United States), which occurred in April 1964. He was infected as a result of working with pipe insulating material. This material contained 75% jute and 25% bovine wool; however, the company subsequently began to use insulation material consisting of 50% bovine wool and 50% imported goat wool. Contact with expressly this material, from which spores of B. anthracis were subsequently isolated in a bacteriological examination, was the cause of the worker's infection ("Report of the U.S. Epidemiological Center, 1964).

There is a direct relationship between the route of infection and localization of the primary anthracic carbuncle. The different parts of the body are ranked in the following order, according to frequency of lesions with cutaneous anthrax: hands, head (cheeks, neck, lips, eyelids, chin), forearms, legs, etc. (A. Ya. Zaporozhchenko, E. N. Shlyakhov).

Most of the anthrax cases are recorded among people in the active employment age: in 1958-1966, 77.5% of those stricken in the Soviet Union were 20-50 years old. There were minor fluctuations in share of such people in the Union republics, from 65.7% in Azerbaijan SSR to 84.4% in Moldavian SSR. This is attributable to the fact that individuals in expressly this age group deal more often than others with farm animals, are involved in slaughtering and dressing carcasses, etc.

The anthrax cases that are still recorded in some republics among infants are related primarily to the distinctions of rural life style, where children are in contact with animals, as well as with livestock products (goat and sheep skins, wool, etc.) used in the homes of livestock farmers and owners of private cattle.

There is prevalence of males among individuals stricken with anthrax. This is attributable to the fact that it is expressly men who are chiefly involved in slaughtering sick animals and cutting up their carcasses, and consequently they are more often submitted to the risk of infection and contraction of anthrax.

The prevalence of infections resulting from direct contact with sick animals or those that died of anthrax also explains why there is marked persistence of summer and fall cases of anthrax among people, due to the seasonal nature of incidence of the disease among animals. The seasonal rise in morbidity of people, like that of farm animals, usually starts in May and reaches a maximum in July and August. The anthrax cases among humans due to the influence of seasonal factors in the summer and autumn months (July-September) constituted over 60% of all cases per year.

There is a similar seasonal nature of incidence of the disease in all parts of our country where anthrax is recorded among people. We find a correlation between the annual morbidity levels and share of cases recorded during the periods of seasonal rises. For example, in the Baltic republics, where isolated cases are recorded, 25-50% are referable to the summer, whereas in central Asian republics, where the morbidity level is higher, 60-70% of the cases are recorded in July-September.

Industrial-occupational anthrax, as well as domestic form thereof, which is unrelated to seasonal morbidity of farm animals, is encountered at any time of the year.

IMMUNOLOGY

Natural insusceptibility: There is rather wide fluctuation of decree of susceptibility to anthrax of different animal species, ranging from virtual absence thereof in birds (species-specific insusceptibility) to a rather high degree in herbivorous animals.

In the opinion of L. V. Gromashevskiy and G. M. Vayndrakh (1947), S. V. Gualits (1958) and others, susceptibility to anthrax is universal, while the low incidence thereof may only be attributed to epidemiological factors, i.e., low infectivity of man.

However, in this regard, there are some interesting observations made in the United States at enterprises processing raw material of animal origin, where cases of pulmonary anthrax occurred among the workers (Brachman et al., 1960; Dahlgern et al., 1960). The stricken workers were those who were invaded by 500 or more dust particles, no more than 5 µm in size, contaminated with anthracic spores, which were capable of penetrating into the alveoli. A survey of these enterprises revealed that the workers in some shops could inhale 600 to 2150 dust particles in the course of an 8-h work day, which were infected with anthracic spores; however, only a few of them were stricken. It is assumed that, in such cases, the difference in individual resistance is significant, so that not all individuals of a species susceptible to anthrax, exposed to the same risk of infection, contract this disease. Evidently, the degree of virulence of the pathogen is also significant (N. N. Ginsburg, 1966).

The factors determining species-specific insusceptibility or individual resistance to anthrax have not yet been definitively established.

Postinfection immunity: Lasting immunity develops as a result of having had anthrax. However, although the cases of repeated anthrax in man are rare, they can occur.

L. A. Rozen'yer (1948) observed a recurrence of cutaneous anthrax after relatively short periods of time (1-3 years). E. N. Shlyakhov (1960) described 3 cases of anthrax that occurred 8-20 years after the first time. K. Kebedzhiyev (1967) observed 3 cases of contracting anthrax twice and 2 cases of contracting it 3 times among 5132 individuals stricken with anthrax over the last 10 years in Bulgaria. Occasionally, the recurrences are associated with severe symptoms.

Postinoculation immunity: Immunity to anthrax may be the result of specific preventive inoculation.

Pasteur, in France (1881), and L. S. Tsenkovskiy, in Russia (1883) proposed the first anthracic strains for specific preventive inoculation of farm animals. The vaccine strains of Tsenkovskiy, from which vaccines were produced, were obtained by culturing the virulent anthracic bacillus at 42° for 12 (first vaccine ["vaccine I"], more attenuated) or 6-7 days (second vaccine ["vaccine II"], less attenuated), and the animals were immunized by administering the vaccine twice. In addition, Tsenkovskiy's vaccine II was used for single inoculation in the form of suspension in a saponin solution (saponin vaccine). At the present time, the vaccines of Pasteur and Tsenkovskiy are virtually unused in agriculture.

The vaccines of Pasteur, Tsenkovskiy and others are attenuated variants of the virulen capsular forms of B. anthracis. Isolation and selection of genetically altered acapsular variants was another route followed by researchers to develop anthrax vaccine. Sterna's live spore vaccine, which is widely used abroad for the prevention of anthrax in agriculture, is such a vaccine, which is derived from acapsular strain 34 F_2.

The development of a highly immunogenic and virtually areactogenic live spore vaccine, STI, played a major role in the control of anthrax among farm animals and humans in the USSR; it was produced on the basis of the vaccine strain, STI-1, obtained in 1940 by N. N. Ginsburg, which is a genetically altered avirulent, acapsular variant of B. anthracis. This strain was developed as a result of isolation and selection from the highly virulent "Krasnaya Niva" [Red Field] strain of a mutant that lost the capacity for capsule formation under laboratory conditions (the strain is named STI after the Sanitary Technical Institute, in which it was developed).

Testing of strain STI-1 (N. N. Ginsburg, R. A. Saltykov, V. R. Arkhipova, 1964) during 18-year storage in dry form and 20-year use thereof in industry revealed that its biological properties were very stable.

During the same years, vaccine strain No 3, which was isolated through breeding by A. L. Tamarin from virulent strain GIEV-III, was obtained by an analogous method in the laboratory headed by N. N. Ginsburg.

In 1950-1955, S. G. Kolesov, N. A. Mikhaylov and Yu. F. Borisovich developed the GNKI vaccine for veterinary purposes; it is a suspension of attenuated strain Shuya-15 precipitated with aluminum hydroxide. In 1960, a method was developed for the production of dry STI and GNKI anthrax vaccines.

The distinctive feature of STI vaccine is that its reactogenicity is extremely low. Already the first trials thereof on farm animals revealed that 1184 out of 2,037,980 inoculated animals presented complications (N. N. Ginsburg, 1946). In the opinion of N. N. Ginsburg (1964), the complications in different species of inoculated animals are parallergies of diverse etiology, which occur due to impairment of the normal physiological state caused by cooling, overheating, carrying pathogens of different infections, etc.

In man, there are virtually no reactions to STI vaccine when administered by the most popular method of application, i.e., epicutaneous (scarification).

We conceive of the mechanism of immunogenesis as a result of specific inoculation of STI vaccine, which was studied by M. A. Chalisov and A. L. Tamarin (1946) in experiments on different animal species, as well as observations of man, as follows: there is rapid vegetation of spores at the site of application of the vaccine, and there is formation of a specific focus, the distinctive feature of which that there is absolutely no necrotic component. From the site of vaccine application, the growing B. anthracis penetrates into regional and remote lymph nodes, spleen, liver and other organs, where it also causes a specific process (data referable to animals).

The protective anthrax antigen formed at the site of reproduction of vaccine cells transforms elements of lymph nodes and spleen into plasma elements, as is the case with formation of antitoxic immunity. Moreover, cellular elements of the macrophage type, which capture vaccine cells, dissolve them, assimilate their antigens, causing rapid conversion of elements of the reticuloendothelial system into microphages with high phagocytic activity and complete phagocytosis (N. N. Ginsburg, 1966).

The development of the STI vaccine made it possible to institute mass scale regular immunization of animals in our country. According to the data of the USSR Ministry of Agriculture, more than 150 million farm animals were inocualted against anthrax in the Soviet Union, in 1966. Annual, scheduled immunization of animals is one of the decisive factors in preventing anthrax in agriculture.

Numerous epizootiological and epidemiological observations are indicative of the high protective efficacy of STI vaccine, for both animals and man, since there are very rare cases of anthrax among those inoculated. According to the official records, people inoculated by the scarification method constituted no more than 2-4% of the anthrax cases, and they had mild cases of the disease (S. M. Smirnov and A. Z. Ter-Karapetyan, 1967). In 1951-1952, a special commission of the USSR Ministry of Health made a study of the epidemiological efficacy of STI vaccine in Moldavian SSR, mainly among the rural population. A total of 141,633 people were inoculated, chiefly by the scarification method. The index of efficacy of the vaccine constituted 5.4 over a 2-year observation period (N. N. Ginsburg, 1966). Introduction of dry and concentrated STI vaccine will enhance even more the epidemiological efficacy of inoculation against anthrax.

Development of a vaccine from protective anthracic antigen is another direction of work on products to be used for immunization against anthrax. As far back as 1904, Bail demonstrated the unquestionable potential of this direction, having established that sterile edematous anthracic fluid could serve as an immunogenic factor.

Forty years later, the work of Gladstone (1946-1948) drew the attention of researchers; he was the first to succeed in recovering protective antigen from an acellular culture of B. anthracis incubated in vitro on a serum medium. The antigen, in a dosage of 0.04 mℓ, protected rabbits against 100 MLD anthracic spore culture when given 3 times at 1-week intervals. These studies settled the question of obtaining anthracic protective antigen in vitro, and they opened the way for practical recovery of vaccine from protective antigen.

In 1951-1955, Wright et al. reported on recovery of protective antigen from a nonproteolytic acapsular strain of B. anthracis cultured on a synthetic medium. The protective antigen contained in the culture filtrate was concentrated and stabilized by precipitation with alum. The immunizing activity of the antigen was established in experiments on rabbits, guinea pigs and monkeys. Schlingmann et al. (1956) experimented with immunization of cattle, sheep and pigs using the protective antigen. The studies revealed that 2 injections of antigen given at an interval of 2 weeks, produced significant immunity against experimental infection. Stable immunity was demonstrable 1 month after the inoculation; its activity diminished after 3 months and was barely noticeable after 8 months. Jackson, Wright and Armstrong (1957) obtained the same results from experiments on cattle.

In 1955-1959, Brachman et al. (1962) tested the epidemiological efficacy of protective antigen at four enterprises that processed goat wool, where cases of anthrax were recorded annually (0.6-1.8 cases per 100 workers). The product was given 3 times at 2-week intervals, in doses of 0.5 mℓ. Subsequently, they were revaccinated twice at a 6-month interval and once after 1 year. A total of 495 people were vaccinated. 414 were given placebo and 340 remained unimmunized. Over a 4-year observation period there were 26 cases of anthrax, including 3 from the immunized group (cutaneous anthrax), 6 from the nonimmunized group (pulmonary form in 3 of these cases) and 17 from the group given placebo (2 cases of pulmonary and 15 of cutaneous anthrax). These authors concluded that the protective antigen had high epidemiological efficacy against cutaneous anthrax.

In view of the brief period of immunity induced by protective antigen, Klein et al. (1962) proposed that antigen vaccination be followed by administration of live vaccine. In their experiments on guinea pigs, such combined vaccination increased resistance to experimental infection by more than 100 million times.

Studies are also being pursued in the Soviet Union to develop protective anthracic antigen, production of vaccines from it and examination thereof (N. I. Aleksandrov et al., 1962). Vaccine strain STI-1 is used for this, as well as original nutrient media (milk with glucose and sodium bicarbonate) and their own concentration method (ethanol). The obtained antigen has been tested on rabbits, guinea pigs and monkeys.

Passive immunity: Hyperimmunization of horses with a mixture of vaccine and virulent strains of B. anthracis makes it possible to obtain "therapeutic" anthrax serum, which induces passive immunity to anthrax when given to man or animals. The mechanism of protective action of this serum cannot yet be fully explained.

Since 1962, therapeutic serum has been replaced in the Soviet Union by anthracic globulin, the specific protective effect of which is related to the beta and gamma globulin fractions.

Allergies: The earliest indication of the possibility of cutaneous allergy in animals infected with anthrax was made by Tsironi (1924). Such phenomena were subsequently observed by Kombiesko, Grushka (1927, 1929), and later by Baltyanu (1943) and others (quoted from E. N. Shlyakhov, 1968). In an attempt to make use of these observations for practical purposes, the veterinarians A. Petrov and Ye. Kiselev (1937) developed and described a method of allergic diagnostics of anthrax infection in animals using the anthraxin preparation they proposed. When testing this product on rabbits, guinea pigs, pigeons and cattle, intracutaneous injection of anthraxin to animals infected with B. anthracis or vaccinated against this infection elicited a cutaneous allergic reaction after 6-12 h in the form of erythema and edema at the injection site. Administration of anthraxin to animals infected with plague or brucellosis, as well as those vaccinated against plague or erysipelas, yielded negative results, i.e., there was no skin reaction. The studies of A. Petrov and Ye. Kiselev demonstrated the specificity of the allergic skin reaction for anthrax, and confirmed its diagnostic value in the presence of this infection.

E. N. Shlyakhov et al. (1957-1964), who made an in-depth study of the phenomenon of cutaneous allergy in the presence of anthrax, discovered the theoretical essence of this effect, which proceeded on the order of delayed hypersensitivity. In 1957, E. N. Shlyakhov proposed a diagnostic product, "tissular anthraxin," and in 1961, together with S. A. Shvarts, a new variant of this product for allergodiagnosis of anthrax, which the authors arbitrarily named "chemical anthraxin." The latter was included in industrial documents and medical practice under the name of "anthraxin." According to the authors' data (1964), anthraxin is a protein-polysaccharide-nucleic complex of B. anthracis. Perhaps, the A-lipid fraction, which remains in the product after removal of lipids that are readily extracted, also plays a certain role.

As with other allergic reactions, when the anthraxin test is positive there is proliferation of histiocytic and lymphoid elements, while the morphological distinction is edema of the subcutaneous tissue (E. N. Shlyakhov, I. G. Shroyt, 1964).

Anthraxin is used widely for the diagnosis of new cases of anthrax in man, as well as retrospective diagnosis of prior illness. The skin tests with anthraxin were positive in 91.5% of the cases 1 month to 30 years after having been stricken with anthrax (E. N. Shlyakhov, S. A. Shvarts, 1966).

The anthraxin test is very important to early detection of new cases of anthrax among humans. Positive tests with anthraxin are observed in 88.5% of those stricken (85% in the first week of illness). If we consider that bacteriological examination prior to the use of antibiotics in medical practice confirmed the diagnosis of anthrax in only 44-52% of the cases, the importance of the anthraxin test for diagnostic purposes becomes obvious.

PATHOGENESIS AND PATHOLOGICAL ANATOMY

The pathogen of anthrax penetrates into man and animals over the most varied routes. Most often, the skin and external mucous membranes are the points of entry in man, while the mucosa of the digestive tract and respiratory tract are the predominant routes in animals.

A distinction is made between cutaneous, pulmonary, intestinal and septic forms of anthrax in man. The intestinal and cutaneous forms are inherent in animals, while many authors deny the existence of the septic form. Animals often suffer from the disease in acute form, and they die soon after infection as a result of development of the apoplectic form.

The cutaneous form of anthrax, which is one of the most common in man, occurs when infection penetrates through skin lesions: scratches, bruises, cuts, abrasions, etc. Exposed parts of the skin are stricken more often than those covered with clothing.

As a rule, the pathogen does not penetrate through areas of skin with profuse superficial fat (nose), tips of the fingers and nail beds.

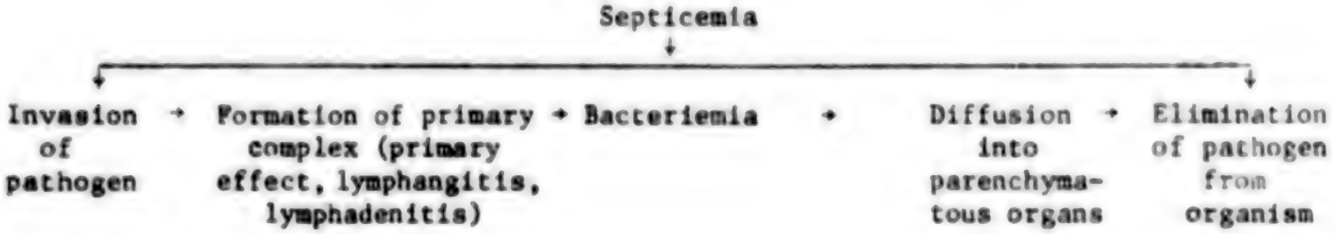
Many aspects of the pathogenesis of anthrax, particularly its visceral forms, have not been sufficiently studied. The mechanism of onset and development of the pathological process can be conceived of as follows: At the site of invasion of B. anthracis in the human skin an anthracic carbuncle appears, which is a unique focus of serous and hemorrhagic inflammation with necrosis, associated with edema of adjacent tissues, hemorrhagic regional lymphadenitis and signs of systemic poisoning. In some cases, when the defenses of the body are impaired, resistance is low and immunity is weak, conditions develop that favor generalization of infection with development of anthracic sepsis. The pathogens penetrate from regional lymph nodes into the general circulation, where they not only remain viable, but continue to multiply. As a result of generalization of infection, secondary hemorrhagic and necrotic lesions are formed. The process evolves into sepsis.

To this day, there is no agreement as to the pathogenesis of intestinal anthrax. Some authors do not consider it to be an independent form, but as secondary, which develops via the hematogenic route. When anthrax spores are introduced into the lungs of animals they are soon demonstrable in the intestine. In those suffering from the cutaneous form of anthrax bacilli are also isolated from the intestinal contents, where they were brought with blood. As a rule, isolated swelling of the cervical lymph nodes is found in animals that died of intestinal anthrax. This warrants the belief that the point of entrance of the infection is the upper gastrointestinal tract: mouth, pharynx and esophagus. Then there is hematogenic passage of B. anthracis into the intestine. Pulmonary anthrax is encountered only in man, and it is not observed in animals. Special significance is attributed to inhalation of dust containing many fine prickly particles, in addition to anthrax spores. This injurious mixture causes invasion of B. anthracis and facilitates development of the disease. The experimental data of a number of authors indicate that inhaled anthracic spores do not proliferate into the lungs, but after being captured by macrophages they are carried with the flow of lymph into the tracheobronchial lymph nodes. There they develop within the phagocytes that captured them, then enter the blood stream and cause generalized infection.

The development of the secondary septic form is considered to be a complication of any of the clinical variants. When there is massive infection and considerable virulence of the pathogen, in the case of inadequate defenses of the macroorganism, the primary septic form of anthrax can develop. There are indications of rare cases of anthrax (anthracic septicemia) without the formation of carbuncles or changes referable to the viscera. The primary septic form is observed, which develops when the bacilli penetrate through the tonsils or respiratory tract, which do not react in the form of any local process, while the blood and spinal fluid contains B. anthracis.

In some cases, any clinical form of anthrax may evolve into specific hemorrhagic meningoencephalitis or leptomeningitis. Cases of primary meningitis have been described, when it was not possible to determine the site of invasion of infection either clinically or anatomically.

V. N. Nikiforov (1960) submits an original scheme of the pathogenesis of anthrax:


Septicemia
Invasion of pathogen
Formation of primary complex (primary effect, lymphangitis, lymphadenitis)
Bacteriemia
Diffusion into parenchymatous organs
Elimination of pathogen from organism

According to the data of different authors, the incidence of different clinical forms of anthrax in man is as follows: cutaneous 95%, visceral 5% (of which 48% is referable to intestinal, 23% to pulmonary and 29% to other visceral lesions).

The clinical signs of anthrax in man and animals, course of illness and its outcome are apparently largely determined by the effects of specific toxins. N. N. Ginsburg (1964) et al. believe that the pathogenicity of B. anthracis is closely related (if not entirely determined by) to two factors, capsule formation and toxin production.

Nor has the question of pathophysiological action of anthrax toxins been definitively answered as yet. In the presence of anthrax, the central nervous system becomes involved in the pathological process as a result of hypoxia (Lincoln, Walker, Klein, Haines, 1964). In particular, the hypothalamus-adrenals axis is stricken, and there is increased function of the latter (adrenal cortex). Degenerative changes are found in the autonomic and sympathetic nervous system of different animals. Some authors believe that the changes in the central nervous system are primary, while others consider them secondary. In the opinion of some authors, death due to apoplexy can be attributed to a lesion to the respiratory center. However, as indicated by Lincoln et al. (1964), there are still not enough convincing data or corroborative studies of the central nervous system.

Blood chemistry changes in the presence of anthrax. Calcium salt deficiency is given a significant place in the pathogenesis of anthrax. Administration of calcium salts to animals protects them against progressive poisoning. A decline of blood oxygen, from 12.4 and 16.5 to 0.2%, has been observed. This was not associated with serious impairment of O_2-binding capacity of hemoglobin. After giving rabbits anthrax toxin, CO_2 content of blood increased from 45 to 60%. There was also an increase in leukocyte count, with a shift to the left of the white cell formula. In Macaca rhesus monkeys, these blood changes were more significant. Erythrocyte sedimentation rate increased from 5 to 35 mm/h. According to some authors, hematocrit did not change. According to Lincoln et al., it dropped from 40 to 19%. The specific gravity of urine did not change. Considerable changes in urea nitrogen were noted at the terminal stages of the disease. Total serum protein and nonprotein nitrogen were in the normal range; plasma pH decreased. Calcium ions decreased while potassium ions increased. There has been little study of changes in other mineral salts. Lincoln et al. (1964) found hyperglycemia in 14 out of 21 rabbits, and a tendency toward hypoglycemia in the Macaca rhesus monkeys.

Pathoanatomical changes: External examination of cadavers shows no rigor mortis due to rapid decomposition of tissues in humans animals that died of anthrax. Autopsies revealed impressive, severe hyperemia of internal organs and plethora thereof. The spleen and liver are enlarged, plethoric and dark red. Hemorrhages are observed on their surface. The myocardium is flaccid. There are hemorrhages in the myocardium and epicardium. There could be tufted proliferations on the valves (due to penetration of B. anthracis). The blood in the chambers of the heart is dark red, like in the veins, fluid, does not clot and contains large quantities of B. anthracis.

The lungs usually demonstrate bronchopneumonic lesions with serous and fibrinous, as well as hemorrhagic saturation of tissues, hepatization and edema, hemorrhages and necrosis of the mucosa. The bronchial and paratracheal lymph nodes markedly enlarged, with hemorrhages; their consistency is soft, they are dark red or even black.

Carbuncles of different sizes, occasionally up to several centimeters in diameter, may form in the intestine. The carbuncular surface is covered with a dirty scab. Subsequently, ulcers surrounded by edematous swelling are formed. In other cases, there are no carbuncles, but there is diffuse hemorrhagic infiltration. Metastatic lesions lead to small hemorrhages, without marked edema around them. There is enlargement of mesentery lymph nodes. They are dark red and contain numerous B. anthracis. There is a significant amount of serous blood exudate in the abdominal cavity, and peritonitis. The brain and meninges are also subject to major pathological changes. The dura mater is taut, plethoric, the sinuses are dilated and contain a large amount of thick dark blood. The pia mater is thickened. It is dark red, its vessels are dilated and plethoric. On sections, the brain tissue is flaccid, the color of raspberries, with many petechial hemorrhages. There are many hemorrhages in subcortical nodes.(could be extensive).

Very severe changes are found in the organs where the pathogen of anthrax entered and multiplied. The nature of these changes varies, depending on the clinical form of anthrax.

With the cutaneous form, a pustule appears at the site of invasion of the pathogen, with serous hemorrhagic contents, then a carbuncle develops in the form of a well-circumsribed swelling of firm and, less often, pasty, consistency. A hard dark brown scab forms in its center. The skin under it is necrotic, while the subcutaneous tissue is saturated with serous hemorrhagic exudate. Furuncles with bloody contents, which subsequently dry up, appear around the scab in the form of a crown. The tissues around the carbuncle are edematous, saturated with serous hemorrhagic exudate with admixture of neutrophils and fibrin. Suppuration or abscesses are never observed (I. Chalisov, 1963). The lymphatic vessels around the carbuncle are dilated (lymphangitis). The regional lymph nodes are enlarged, edematous, of a soft consistency, and they are often dark red due to hemorrhagic infiltration. B. anthracis are found in considerable quantities in the contents of pustules, edematous fluid and lymphatic vessels. They are not demonstrable on the surface of necrotic areas. Less often, they are found within the surface layer of the crust, mostly in degenerated forms, whereas in the deeper layers they present the typical form. In scabs that are several days old there are no bacilli. It must be noted that phagocytosis of B. anthracis by neutrophils and histiocytes is not observed in tissues and body fluids. Occasionally, cutaneous anthrax occurs without a primary carbuncle, in the form of acute, diffuse and inflammatory edema and marked sepsis (I. Chalisov, 1963).

In the case of intestinal anthrax, the lesions are localized mainly in the lower segment of the small intestine, near Bauhin's valve and in the cecum. Most often they are isolated, but they may be present in quantities of up to 20-30. The lesions vary in size, whereas their structure is analogous to that of a cutaneous carbuncle. The retroperitoneal tissue, mesentery lymph nodes, omentum and mesentery are saturated with blood. Other parts of the gastrointestinal tract are involved less often than the small intestine. When there is secondary involvement of the intestine via the hematogenic route, one encounters small hemorrhagic sites without edematous swelling and in the form of numerous edematous hemorrhages, often scattered in various parts of the small intestine, duodenum and stomach.

There can also be other variants of lesions: diffuse hemorrhagic enteritis, multiple intestinal carbuncles and the ulcerative form. The primary intestinal form of anthrax usually evolves into anthracic sepsis.

With the pulmonary form of anthrax, numerous pneumonic lesions are found in the lungs, which take up a significant part of the stricken organ. There are hemorrhages, bloody infarcts and gangrenous lesions in different parts of the lungs. The alveoli contain a serous exudate, while interstitial tissue demonstrates serous-gelatinous infiltration. The paratracheal and bronchial lymph nodes are enlarged, plethoric, soft, dark red and sometimes black. There is serous or serous-hemorrhagic exudate in the pleural cavity. As a rule, primary pulmonary anthrax evolves into sepsis.

Histological changes: There are significant changes in the small vessels, and less often in larger ones. A distinction is made between primary and secondary lesions. In the case of primary processes in vessels, the lesions develop mainly from the outside in. Necrosis spreads from the adventitia in the direction of the intima. In the case of secondary lesions that occur hematogenically, the vessels are dilated, plethoric and stasis thereof is observed. Conversely, it is the intima that is subject to necrosis, but all layers may be necrotic, and formed blood elements penetrate into the tissue surrounding the vessel. The vascular endothelium is swollen, enlarged and presents desquamation. The spleen is overfilled with erythrocytes and flaccid. The intima of many large vessels is necrotic. Extensive hemorrhages are found.

In the liver there are dystrophic changes and signs of acute nonsuppurative hepatitis. The intima and, occasionally the media of vessels are necrotic; however, there are no changes in the adventitia, which explains the lack of hemorrhages in the liver.

Very severe changes with signs of distinctive encephalitis are observed in the brain. There are numerous hemorrhages in brain tissue due to the severe lesions to vessels. The process occurs in the form of leptomeningitis in the pia mater, most often hemorrhagic and less often serous. Vascular necrosis is in the nature of coagulation with subsequent colliquation. One encounters vessels all of whose walls are entirely destroyed, for which reason there are extensive hemorrhages into the pia mater, that becomes hard to distinguish.

Histological examination of the intestinal wall shows it to be edematous. All of its layers present separation of fibers, and they are infiltrated with many formed blood elements. There are major changes in the large lymphatic and blood vessels. They are compressed and destroyed in the submucosal layer. This is associated with impaired nutrition of the epithelial layer, and it becomes necrotic. Large quantities of B. anthracis can be isolated from the submucosal layer. Histological examination of lymph nodes reveals acute hemorrhagic lymphadenitis.

The pathoanatomical changes in animals are essentially quite similar to those demonstrated in man. The carcasses are distended, there is mild or no rigor mortis. The blood in vessels is dark, almost black, and thick. Tissues around lesions are edematous, gelatinous and present numerous hemorrhages. The regional lymph nodes are enlarged, plethoric, with extensive hemorrhages. There is plethora of all internal organs, as well as dystrophic changes septic in appearance in the liver and spleen, with multiple sites of hemorrhagic pneumonia in the lungs, effusion of blood on the mucosa of the gastrointestinal tract. There are areas of edema or focal lesions to the lymphatic system that resemble anthracic carbuncles. In the case of the instantaneous (apoplectic) form of anthrax, which frequently occurs in animals, the typical morphological signs of the disease may not be found upon post mortem examination. It must be recalled that B. anthracis is isolated from blood and all organs, and it is demonstrable in smears.

The course of anthrax in pigs is somewhat unique. The process is usually limited to the pharyngeal region, where acute serous-hemorrhagic inflammation is observed, with severe edema of adjacent tissues. Pathoanatomical changes are not necessarily present with the apoplectic form of anthrax.

SYMPTOMATOLOGY

Polymorphism of anthrax symptoms is typical, and it depends largely on the route of penetration of the pathogen. As we have already mentioned, the disease occurs in cutaneous, pulmonary, intestinal and septic forms. There is little justification for singling out the meningeal form with a marked meningeal set of symptoms, which may develop in patients. This form could be referred to the complications of septic or other form of anthrax.

The incubation period of anthrax is brief, an average of 2-3 days, ranging from several hours (intestinal, pulmonary and septic forms) to 6-8 days.

The cutaneous form is characterized by development of distinctive specific carbuncles and signs of general poisoning. As a rule, there is one carbuncle, but occasionally the same patient could have 10-20 or more. The cutaneous form of anthrax is the most frequent one (95% of the cases).

After the incubation period of cutaneous anthrax, the following appear successively at the point of entry: spot, papule, vesicle, pustule, ulcer. The reddish or bluish spot very rapidly (within a few hours) changes into a copper-red papule, occasionally with a reddish-purple cast, which is raised above the skin level. Local itching and mild burning are observed, which gradually become more severe. The spot is 1-3 mm in diameter. It is very similar to the trace of a flea bite, for which reason French peasants call the disease the "malignant flea" ("puce maligne"). After 12-24 h, the papule changes into a vesicle 2-3 mm in diameter with serous contents, which then become dark, bloody and occasionally reddish purple-violet (pustula maligna). Because of the severe itching, patients often open the vesicle, and it opens spontaneously less often. The walls of the vesicle collapse, a small ulcer is formed, then a crust that turns black rapidly and increases in size. There is a pronounced red border around the crust. The appearance of such a lesion resembles a small piece of black coal on a red background, which is the origin of the name of this disease (from the Greek anthrax--coal).

Usually there is an eruption of secondary pustules around the crust, which are very similar to the primary pustule. They are located around the central region occupied by the scab. The appearance of the lesion resembles a black diamond surrounded by small pearls. These pustules undergo the same developmental stages, and the bursting vesicles enlarge the crust, i.e., small crusts are joined together into a black-brown or entirely black large crust up to 5-6 cm in cross section in some cases. The crust is usually round or peculiarly elongated, but more often oval-round. The scab is firm, like a very brown or burned crust, often slightly concave and uneven (G. P. Rudnev). An infiltrate develops under the scab in the form of an uneven ridge rising above the level of healthy skin.

Edema is the second most important diagnostic sign; it sometimes involves large areas around the lesion, for example, the entire hand, leg, face. The edematous tissue is pale, pasty or firm, unlike erysipelas inflammation, with no luster. There is severe edema in areas of friable subcutaneous tissue, especially on the face, and the patients cannot open their eyes (Figures 5 and 6). According to the observation of G. P. Rudnev, in one patient the edema involved both sides of the upper shoulder girdle in a distinctive half-scarf fashion, in addition to the head on which there was an ulcer near the ear. When a carbuncle is localized on the lip, there may be edema of the oral mucosa, tongue, extending to the upper respiratory tract, which could lead to asphyxia and death. The extensiveness and spread of edema determine the severity of illness to some extent. Local necrosis may develop if there is severe edema.

Figure 5.
Cutaneous anthrax of the face, crust is beginning to form (after G. P. Rudnev)

Figure 6.
Cutaneous form of anthrax of the left eyelid (scab formation and severe edema)

Upon tapping the regions of edema with a percussion hammer, there is gelatinous quivering, which could be used as a diagnostic symptom (V. K. Stefanskiy). Local anesthesia is an important diagnostic sign. There is virtually no pain in the involved regions, merely contact is perceived. Needle-pricks into the ulcer or its vicinity are painless, and this is a valuable sign in differential diagnosis of cutaneous plague and tularemia. In the latter cases, there is much outward similarity of local lesions, but they are extremely painful.

A three-color transition from the middle to the periphery is inherent in cutaneous anthrax (according to G. P. Rudnev): the black scab in the middle, then a narrow suppurative yellowish band and, finally the wide reddish-purple ridge (the intermediate yellowish suppurative band is lacking with cutaneous form of plague).

Finally, with the cutaneous form of anthrax, there is subsequent addition of regional lymphadenitits, and the severer the course of illness, the more marked it is, combining with lymphangitis. The enlarged lymph nodes are slightly tender.

The patient's general condition and well-being are not impaired in the early hours after appearance of local changes, but there is general malaise, fatigue and headache. By the end of the first day, or on the 2d day of illness, signs of systemic poisoning appear. Temperature rises to 39-40" with moderate chills. There is worsening of the patients' general condition, appearance of general weakness, headache, vertigo, poor appetite; there may be nausea, vomiting, elevation or drop of arterial pressure. The fever lasts for 5-6 days, then the temperature drops critically (Figure 7). In most cases, there is a complete recovery with rapid improvement of the patients' general condition. The temperature drop coincides with disappearance of signs of intoxication and regression of local manifestations of the disease. There is gradual decrease in edema, disappearance of lymphadenitis and lymphangitis. However, the local signs regress with relative slowness. The scab is sloughed off by the end of the 2d-3d week, leaving a cicatrix (V. N. Nikiforov, 1960).

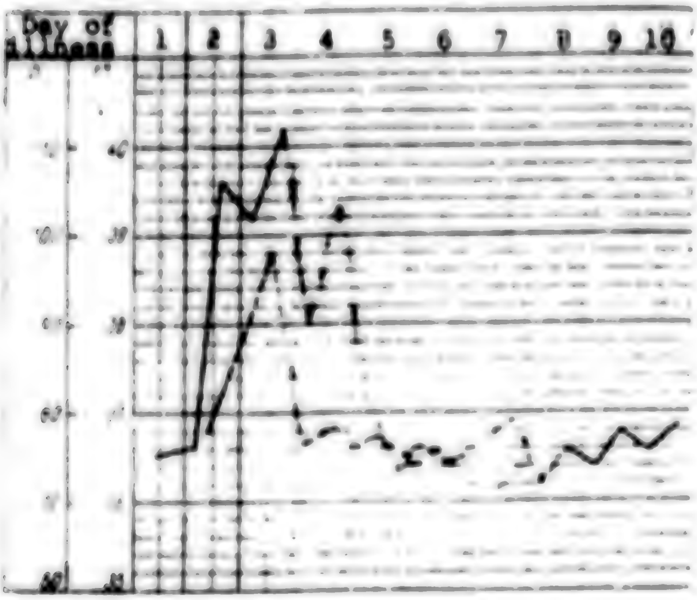


Figure 7.
Typical temperature curve in the presence of anthrax

In some cases, at the stage of convalescence after decline of temperature, there is sudden worsening of the course of illness due to development of the secondary septic form. Temperature rises over a wide range, and chills recur. There is considerable intensification of headaches, with appearance of bloody vomiting in some cases, with debilitating bloody diarrhea as well. Pulse rate increases and arterial pressure drops. Upon palpation, the liver and spleen are enlarged. There may be secondary pustulous and other eruptions on the skin of hematogenic-metastatic origin. Severe endocarditis develops.

The patients become excited, restless, and they develop dyspnea and cyanosis. Their condition progressively worsens, the pulse becomes thready and fast (up to 120-160/min) or is not palpable at all. Pulmonary edema develops. The body is covered with large drops of perspiration, sopor occurs, followed by coma and death. The patients often expire without losing consciousness.

In some cases, a carbuncle is not formed at the point of entry of infection. This form, which is called "malignant edema," is usually fatal. The secondary septic form may be associated with secondary microflora (Staphylococcus, Streptococcus). In some cases, the edematous region at the site of a skin ulcer is intensively hyperemic, resembling erysipelous inflammation. This served as grounds to single out the erysipelas-like form of anthrax, which is considered unjustified (G. P. Rudnev).

With the typical course of cutaneous anthrax, moderate leukocytosis is observed with a slight left shift, whereas in the presence of sepsis, on the contrary, there are considerable changes in the leukogram.

The outcome of cutaneous anthrax depends on the condition of the macroorganism and localization of the carbuncle. The outcome is poor when the lesions are on the face, head or neck. Virulence of the pathogen, time and method of treatment are significant. The prognosis is poor in cases of development of sepsis.

The clinical signs of intestinal anthrax are very diverse, unlike cutaneous anthrax. In some cases, manifestations of systemic poisoning are prominent, in others there is prevalence of local symptoms referable to the gastrointestinal tract. There is a sudden onset to the disease, and it develops rapidly thereafter. There are very severe systemic signs; at first the temperature may be subfebrile, but it then rapidly reaches high figures (39-40°). The patients present severe chills, headache, vertigo, general weakness, pain in the lumbar region. Typically there is acute, cutting pain in the abdomen localized mainly at the bottom of the abdomen and less often in the region of the gallbladder. Not infrequently, such patients are admitted to the surgical department with the diagnosis of acute abdomen, peritonitis or intestinal obstruction. Nausea, bloody vomiting with bile and then bloody diarrhea soon develop; intestinal paresis may occur. Anthracic lesion to the intestine leads to irritation of the peritoneum, exudate and, later on, perforation and peritonitis (G. P. Rudnev).

The liver and spleen become enlarged. There is marked weakness of cardiovascular function. The pulse is fast, with poor filling, irregular and, later on, thready. Arterial pressure is very low. Auscultation reveals many static rales in the lungs. There is rapid progression of cyanosis, as well as dyspnea. Secondary pustulous and hemorrhagic eruptions appear often on the skin.

The patient is excited, restless, delirious and, not infrequently, euphoric. Toward the end of the illness there may be seizures, loss of consciousness and meningeal signs. The illness usually lasts 3-4 days, but sometimes death occurs earlier, with signs of progressive weakness of the cardiovascular system. Recovery is very seldom observed from this clinical form. In such cases, the disease acquires a protracted course, there is gradual regression of symptoms and the patient slowly recovers.

The course of primary pulmonary anthrax, like that of intestinal anthrax, is very rapid and extremely severe. After brief incubation, the illness has an acute onset with intensive chills and temperature elevation to 39-40°. Patients complain of a constricted feeling in the chest and cough. Rhinitis and lacrimation appear. Signs of systemic poisoning progress very rapidly: extreme weakness, headache, vomiting, tachycardia, drop of arterial pressure, cyanosis, dyspnea, profuse perspiration, delirium and seizures.

The developing pneumonia is in the nature of acute pulmonary edema. Most often it is bilateral, of the lobular pneumonia type. Coughing produces significant amounts of foamy liquid sputum with admixture of blood. The produced sputum coagulates, and it resembles raspberry jello. It contains large quantities of B. anthracis. Percussion may reveal focal dullness and changes that are very similar to those observed with severe bronchopneumonia. In most cases, auscultation reveals a profusion of dry and, particular, moist fine to large bubbling rales. There is roentgenological confirmation of presence of exudate in the pleural cavity. The fluid obtained from puncture of the pleural cavity is usually hemorrhagic.

The patient's condition worsens rapidly, and there is intensification of dyspnea and cyanosis. Pulse is difficult to palpate, there is catastrophic drop of arterial pressure and heart sounds are dull. The patient expires on the 2d-3d day with signs of collapse and edema of the lungs. In some cases, as with intestinal anthrax, there is prevalence of signs of systemic poisoning. The disease progresses extremely rapidly, with no time for edema and other signs referable to the lungs to develop, and the patient expires within the first day [or days].

Aside from secondary septic anthrax, which is the consequence of other clinical forms, there may be generalization without initial local lesions on the skin. This primary septic form occurs when there is low resistance of the macroorganism, high virulence of B. anthracis, which penetrates into the body through the mucosa.

The course of disease is rapid, extremely severe, with a profusion of hemorrhagic manifestations on the skin and large quantities of pathogen in blood and spinal fluid. Death usually occurs on the 1st day, less often on the 2d day of illness.

Anthrax in animals occurs in the intestinal and cutaneous forms. Many authors deny the existence of the primary septic form. The incubation period lasts 1-3 days, sometimes less. Anthrax may have an instantaneous (apoplectic), acute and subacute course (S. N. Vyshelesskiy and F. A. Terent'yev, 1954). With the apoplectic form, that is more often observed in sheep and goats, the animals become sick suddenly. They become excited, present dyspnea, discharge of bloody foam from the nose and mouth, dark colored blood from the rectum, and a few minutes after appearance of the first symptoms the animal usually drops to the ground in convulsions and dies.

When the course is acute, the animals' temperature rises to 41-42°, breathing is labored and fast, and they lose their appetite. The intestine is distended, feces and urine are bloody. In horses, the disease is associated with colic attacks. In pregnant cows, miscarriages are observed. At the agonal stage, which usually occurs after 2-16 h, there is appearance of seizures, dyspnea, and the animals die presenting signs of asphyxia.

With the subacute course of the disease, which is more often observed in cattle, the clinical signs are the same as with the acute form; however, there is alternate intensification and attenuation of symptoms. The subacute form lasts an average of 48 h, 5-7 days at most.

In pigs, anthrax occurs in most cases in the anginose form of the disease. Body temperature rises to 40-41°, and vomiting is observed. The pharyngeal lymph nodes are enlarged, and there is a diffuse inflammatory edema of the submaxillary and parotid glands extending to the chin, chest and abdomen. Death occurs with signs of asphyxia. Anthrax with visible local skin lesions (carbuncular form) lasts an average of 3-7 days. The carbuncular form may be associated with acute or subacute course of anthrax, but it may also occur as an independent form. Not infrequently, the animals recover when carbuncles are present.

DIAGNOSIS AND DIFFERENTIAL DIAGNOSTICS

The diagnosis of anthrax is made on the basis of the clinical signs, epizootiological and epidemiological data (patient's occupation), and it is based mostly on the results of positive laboratory tests for demonstration of the pathogen. The absence of clearcut epizootiological and epidemiological data creates considerable difficulties in establishing the diagnosis of anthrax in man and animals, and this applies in particular to diagnosis of visceral forms of the disease.

Differential diagnosis is made with regard to such diseases as glanders, plague, benign furuncles, carbuncles, pneumonia, tularemia, sepsis of diverse etiology, meningitis, etc. There is no difficulty in diagnosing uncomplicated cases of cutaneous anthrax. The presence of development of a typical anthracic carbuncle, significant edema, absence of tenderness of the involved area, lack of suppuration, moderate fever, negligible signs of poisoning and even absence of the latter for the first few days make it easy to identify this disease.

Furuncles and benign carbuncles are notable for significant painfulness, inflammatory reaction of surrounding tissues, moderate edema of the latter and suppuration. Erysipelas is characterized by a rapid course of illness from the very first days, temperature elevation and chills. Typically, the edema is circumscribed and contained in a ridge. The lesions are red, taut and very painful, which is not typical of anthrax. White erysipelas presents considerable difficulties, especially when it occurs as a result of burns. In such cases, there is a dark crust, white swelling, tender ridge around the lesion.

Occasionally, the local changes are confused with insect bites. Identification is aided by the history and relatively rapid appearance of edema at the site of the bite. If this site is rubbed with cotton saturated in aqua ammonia, a brownish spot appears.

Glanders starts with a moderate temperature elevation, which is rarely associated with chills. Typically there is pain in the bones, joints and muscles. Numerous and deep abscesses often form in the latter. The integumental manifestations of glanders are notable for tenderness (but not always!), with numerous mildly edematous areas and absence of black crust. Occasionally the lack of tenderness of glanders nodules on the skin and presence of very marked lymphangitis are somewhat similar. However, the secondary eruptions on the skin are more numerous with glanders (tens and even hundreds) than with anthrax. Rhinitis, more often unilateral, is very typical. In the presence of glanders-related pneumonia, the sputum contains blood, but there is much less blood than with anthrax. It is more viscous and thicker, rather than foamy and liquid.

Cutaneous anthrax may resemble the same form of plague, but with the former one observes predominant localization on exposed areas. The lack of tenderness of skin lesions is very typical of anthrax, but is present with plague. The patients often shout out, even when one touches a plague bubo or cutaneous ulcer. One must bear in mind the eruption of secondary vesicles around the crust in the case of anthrax. The crust itself is darker or even black. In addition, severe edema of adjacent tissues and lymphangitis are typical. However, regional lymphadenitis is observed somewhat less often than with plague.

Pulmonary anthrax may be confused with the same form of plague. The patients' general condition with these forms is extremely serious, there are signs of pneumonia, discharge of bloody sputum, the disease progresses and rapidly leads to death. The following data referable to anthrax help make the distinction: catarrhal signs referable to the upper respiratory tract at the outset (rhinitis, hoarseness, salivation). There is a relative profusion of percussive and, especially, auscultative findings in the presence of anthracic pneumonia, which are minimal and vague in the case of plague-related pneumonia. The epidemiological history, as well as results of laboratory tests and the allergy test with pestin are helpful in diagnostication.

Large amounts of serous-hemorrhagic sputum are not observed in cases of pneumonia and bronchopneumonia of any other etiology. The patients' condition is not as serious, the signs of systemic intoxication are much less marked than with anthrax. The course of illness with ordinary pneumonia is benign in most cases.

A differential diagnosis of septic forms of anthrax must also be made with reference to sepsis of diverse etiology. Detection of a focus of infection in the presence of sepsis, epidemiological and laboratory findings are helpful in making the diagnosis.

It is necessary to differentiate between anthrax and tularemia, particularly the cutaneous-bubonic or glandular-ulcerative form of tularemia. In the presence of the latter, as with anthrax, one can successively find a spot, papule, vesicle, pustle and ulcer. The tularemic ulcers heal slowly, and usually by means of cicatrization; they are extremely painful, and there is very marked regional lymphadenitis (bubo). The ulcers are smaller than with anthrax. There is no black crust or edema of surrounding tissues in the presence of tularemia.

The patients' general condition is relatively better than with anthrax. The signs of systemic poisoning are less marked, there is no malignant course of illness, which occurs with the visceral forms of anthrax. The outcome of tularemia is favorable. The febrile period extends from 5-7 to 30 days, while a long period of subfebrile temperature is typical in the recovery period.

However, one must bear in mind that, in the case of pneumonia associated with tularemia, it is sluggish and depleting, without cycles, and it lasts 2 or more months. The production of bloody sputum is a sign in common between tularemic and anthracic pneumonia, but it is observed much less often than with anthrax and plague (G. P. Rudnev). Pulmonary anthrax has a rapid intensive course, and the patient expires within the first few days. The allergy skin test with tularin helps make the diagnosis.

Intestinal anthrax and the abdominal form of tularemia have a similar clinical course (particularly with regard to nature of pain). The abdominal pain may be so severe that it resembles the signs of acute abdomen. This circumstance could even lead to surgical intervention.

The brief, intensive course and lack of clearcut clinical signs of the disease make it difficult to make the diagnosis of intestinal anthrax while the victim is alive. This form is often diagnosed as sepsis, severe dysentery, peritonitis, acute abdomen, intestinal obstruction, meningitis, etc. A thorough clinical examination of the patient, with mandatory consideration of epidemiological history and, particularly, positive bacteriological findings help make the correct diagnosis.

Table 5.

Elements of local reaction		Evaluation of reaction
after 24 hours	after 38 hours	
No infiltrate (possible hyperemia)	No reaction	Negative (-)
Hyperemia and infiltrate over 8 mm in diameter	Hyperemia less than 8 mm in diameter	Doubtful (±)
Hyperemia and infiltrate over 8 mm in diameter	Hyperemia, or hyperemia with infiltrate over 8 mm in diam	Positive (from + to ++++)*

*The intensity of positive reaction is determined from the diameter of hyperemia after 24 h and definitively after 48 h:
up to 15 mm--mildly positive (+)
16 to 25 mm--positive (++)
26 to 30 mm--strongly positive (+++)
over 41 mm--strongly positive (++++)

Allergological diagnostics: The anthraxin method of diagnosing anthrax is based on the response of a patient who has had or was immunized against anthrax in the form of hyperemia and infiltrate, as a local allergic reaction to intracutaneous administration of the product. This capacity may appear in the early days of illness and persist in former anthrax victims for several years.

Method of intracutaneous test: The test is made on the internal aspect of the forearm. Anthrax is injected strictly intracutaneously in a dosage of 0.1 mℓ with adherence to aseptic rules. A control test is made on the other forearm (0.1 mℓ isotonic sodium chloride solution). The reaction is graded after 24 and 48 h using the scale shown in Table 5.

Formation of hyperemia and infiltrate larger than 8 mm in diameter at the site of injection of saline justifies cancellation of the test. If a doubtful reaction is obtained, the anthraxin test should be repeated after 5-7 days. Anthraxin must be clear and should not contain any suspended particles; the vials should not have any cracks. A product that does not conform with these conditions cannot be used. The shelf life of anthraxin is 1 year from the time it is produced, provided it is stored in a dark dry place at a temperature of 4-10°. If the shelf life has expired, the product is not to be rechecked.

When using the anthraxin test, it must be borne in mind that a positive reaction could be the result of prior anthrax or immunization against this infection and, consequently, could have no bearing on the existing disease. Nonspecific reactions to anthraxin are observed in about 5% of the cases. On the other hand, the anthraxin test could be negative in a patient with anthrax when antibiotic therapy is instituted at an early time (E. N. Shlyakhov, 1964). For this reason, one should evaluate the results of the skin test with anthraxin for identification of anthrax only in conjunction with all of the clinical, epidemiological and laboratory findings.

TREATMENT

Treatment of all clinical forms of anthrax must be complex, systemic and, for the cutaneous form, local as well. Treatment is subdivided as well into specific and nonspecific (G. P. Rudnev). There is a history to treatment of the disease, and a large number of various therapeutic agents had been recommended at one time.

Serotherapy, which was proposed by Sklavo in 1895 and somewhat later by Marchu, has stood the test of time, resulting in significant (to 3%) decline of lethality, especially when large doses were given. For a long time, treatment with specific anthrax serum was the most important method, which had an antitoxic action. The efficacy of serotherapy was also confirmed in experiments on animals. Smith et al. (1955) demonstrated that administration of serum to guinea pigs neutralized the edema factor. Large doses of serum prevented death among monkeys (Klein et al., 1962). Lincoln et al. (1964) reported that, regardless of the mode of administration of therapeutic serum (intravenous or intramuscular), the effect was the same. The authors also referred to a more effective combination, serum and antibiotics. Under such conditions, a larger number of infected animals survived.

After preheating to 35-37°, anthrax serum was injected hypodermically or intramuscularly. Intravenous infusion of serum was not desirable due to the development of severe serum sickness (P. N. Burgasov). Adults were given 60 to 100-200 mℓ at a time, whereas later on, after 1-2 days, serum was given again until there was clinical imporvement of the patient's condition, depending on the severity of the disease and its course. The effect of serotherapy was manifested by an improvement of the patients' general condition and regression of signs of intoxication. Serum was used for preventive purposes in doses of 25-50 mℓ when there were epidemiological indications related to a special risk.

We submit below excerpts from the case histories of patients under treatment by A. I. Kolobkova (1950).

> Patient B, 53 years of age, was admitted to the hospital on the 4th day of illness, complaining of general weakness and facial edema. The patient discovered a "pimple" on his right cheek, which grew rapidly in size, and edema of the cheek developed. On the next days, weakness progressed, edema increased, temperature rose to 38°, which is what prompted the patient to see a physician who referred him to the hospital, with the diagnosis of anthrax.
>
> When admitted, the findings are: pustule the size of a 5-kopek coin on the left [sic] cheek, in the region of the mandibular angle. There is

> a crown of fine vesicles with hemorrhagic contents around it. Edema of the cheek. Regional lymph nodes are enlarged to the size of a hazelnut, without tenderness. Anthrax vaccine in a dosage of 150 mℓ was given intramuscularly upon admission. On the following day edema spread down on the neck to the clavicle. Another 200 mℓ anthrax serum was given on the 5th and 6th days, and cardiac agents were prescribed. A crust developed on the 6th day. Edema disappeared by the 10th day. The crust was sloughed off on the 28th day, followed by formation of a secondary scab that fell off on the 41st day. Complete cicatrization of the ulcer on the 47th day of illness. A culture of pustular contents revealed B. anthracis. The patient recovered.
>
> Patient B, 50 years old, had a carbuncle on the left side of the neck. Upon admission on the 2d day of illness his temperature was 38.3°. There is a pustule, the size of a 2-kopek coin on the neck. Edema of the neck and anterior chest wall. Subsequently, edema spread to the abdomen and head. Regional lymph nodes are enlarged, without tenderness. The spleen is enlarged. Dry and moist rales in the lungs. Serum was given for 4 days, in doses of 20 mℓ daily. The patient's condition improved on the 7th day of illness. He was discharged in good condition on the 72d day.

In the last few years, anthrax serum was replaced with anthracic globulin. Anthrax globulin is derived from hyperimmune horse serum, and it is a 10% solution of specific protein (gamma and beta globulin fractions).

We submit a description of a case of anthrax seen by one of these authors, where the patient was given anthracic globulin.

> Patient D, 72 years of age, was taken to one of the rayon hospitals of Kirgiz SSR on 30 August 1968 in extremely serious condition. Complains of headache, general weakness, dyspnea, cough, ulcer over the mandible, on the right. The patient considers himself to have been sick for about 3 days. There was an acute onset. A red, itching spot appeared on the skin over the right mandible, and a vesicle filled with serous liquid appeared at the site of the spot in the evening. The vesicle was opened by the patient, and in its place an ulcer developed with a dirty-gray fundus. Soon there was edema of subcutaneous tissue around the carbuncle, which extended to the cervical region and chest after 1 day. By this time, i.e., on the 3r [illegible] the patient was hospitalized. He had not received preventive inoculations against anthrax.
>
> Objective examination revealed that his condition was very serious, temperature 38°. The patient is excited, thrashes about, tries to get up and feels pressure in the chest. His pupils are dilated and there is an expression of fear in his eyes. Over the right mandible there is an anthracic carbuncle, 1×1 cm in size, with a necrotic rod in the middle. The carbuncle is surrounded by daughter vesicles, and 2 cm away from it there is a vesicle filled with serous-hemorrhagic fluid. Edema of cervical tissue extends to the chest; it is more marked on the left and descends to the inguinal region. The neck and chest are hypermic. Submaxillary and cervical lymph nodes are enlarged, no tenderness upon palpation. The patient's position in bed is halfway sitting up. Heart margins are

widened in cross section. Heart sounds are not heard. Pulse is thready, 120 per min. The tongue is dry and coated with a white film. The abdomen is somewhat distended, tense and tender to palpation. The liver protrudes 2 cm beyond the costal arch. Spleen is not enlarged. There are no meningeal symptoms. The patient is conscious and answers questions correctly.

The following diagnosis is made on the basis of the acute onset of illness, gradual development of lesions on the skin, presence of carbuncle with enormous edema of subcutaneous tissue and marked intoxication: cutaneous anthrax with localization of carbuncle on the right mandible, serious course, anthracic sepsis.

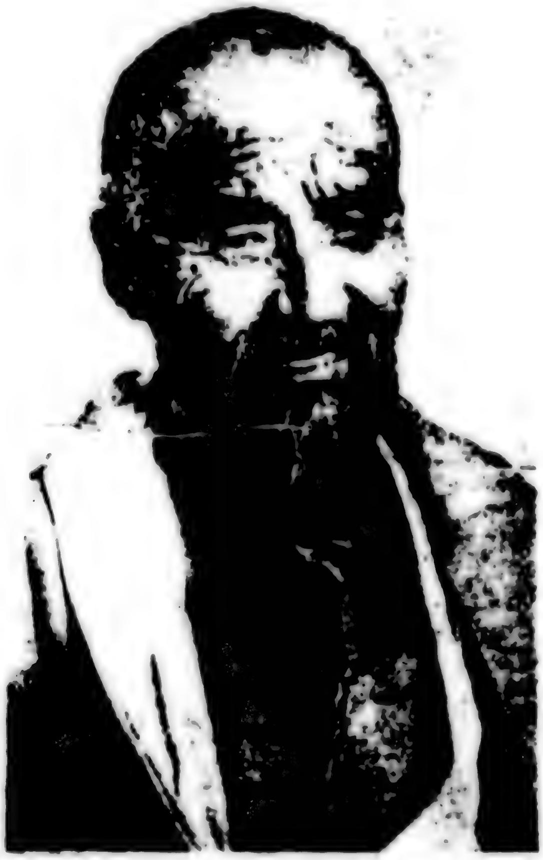


Figure 8. Anthrax carbuncle in patient D. (photo by B. L. Cherkasskiy)

The subsequent course of the disease was extremely serious, with periods of improvement and worsening. There were signs of cardiovascular weakness. Dry and moist rales were found in the lungs. Edema of subcutaneous tissue of the neck and anterior chest wall persisted for a long time. On the following days, an extensive necrotic anthracic ulcer developed on the neck and chest (Figure 8). Healing was slow, with sluggish granulation

> and formation of an extensive black anthracic crust that was firmly attached to the base of the ulcer.
>
> Massive cardiovascular therapy, penicillin therapy, detoxification therapy (polyglucin, glucose) and symptomatic therapy were administered. The patient was given anthrax globulin for the first 7 days (starting with 90 mℓ and decreasing the dosage daily to 30-20 mℓ).

The above case is of definite interest, being one that was characterized by a serious and protracted course of illness, with extensive anthracic lesions to the skin, marked signs of generalized intoxication, but which ended in recovery. In our opinion, the success of treatment is largely related to early administration of anthrax globulin.

The therapeutic dosage of globulin is 30-50 mℓ. If necessary, it is given repeatedly in the same dosage. Preheated (not higher than 40°) globulin is injected intramuscularly in the gluteal region.

An intracutaneous skin test is made to determine individual sensitivity to equine protein prior to administration of globulin, in order to prevent anaphylactic reactions. Therapeutic globulin (10% protein) diluted by 100 times in saline is used for the intracutaneous test. It is injected in the flexor surface of the forearm, intracutaneously under strictly aseptic conditions, in a dosage of 0.1 mℓ dilute globulin, observing the reaction for 20 min. The test is considered negative if the diameter of the papule at the injection site does not exceed 0.9 cm and positive if it increases to 1-3 cm or more in diameter and is surrounded by a zone of erythema.

When the intracutaneous test is negative, administration of globulin is started with a hypodermic injection of 0.1 mℓ, and in the absence of a visible reaction the rest of the prescribed dose is given intramuscularly after 0.5-1 h.

If the intracutaneous test is positive, globulin is given only if there is an immediate threat to the patient's life. The injection is given under the supervision of a physician and taking special precautions. First, dilute globulin is given hypodermically in doses of 0.5, 2 and 5 mℓ at 15-20 min intervals. If there is no reaction to these doses, 0.1 mℓ of undiluted globulin is given. If there continues to be no reaction, the prescribed dose of globulin is given after 30 min. If a reaction appears in response to the small doses, globulin is either not given at all, or given under anesthesia.

If there are signs of an anaphylactic reaction 0.3-1 mℓ epinephrine or 0.2-1 mℓ ephedrine (depending on the patient's age) is given hypodermically, and in serious cases intravenously. Intravenous infusion of 1-8 mℓ 0.5% novocain solution, 3-10 mℓ of 10% calcium chloride, 20-50% glucose solution, use of oxygen and cardiac agents are recommended. In the presence of severe shock, slow intravenous infusion of saline with addition of 0.3-0.5 mℓ epinephrine, 1:1000, is also indicated.

Administration of globulin is combined with other therapeutic methods, in particular administration of antibiotics.

At the present time, there has already been sufficient experience with the use of antibiotics to treat anthrax. As far back as 1945, G. P. Rudnev and A. I. Kolobkova observed the good therapeutic effect of penicillin, which was the most effective when treatment was instituted early and even better when combined with serotherapy and neoarsphenamine. The daily dosage of penicillin given intramuscularly is 500,000-800,000 units or more, depending on the severity of the clinical signs (up to 5,000,000-12,000,000 units per course of therapy).

The results of experimental studies of G. P. Belikov et al. (1956) are also indicative of the efficacy of penicillin and other antibiotics. Penicillin saved over half the animals infected with anthrax from death, synthomycin saved one-quarter, and with administration of biomycin all mice survived. However, it is noted in some reports that antibiotics given to anthrax-infected animals postpone death, but in most cases do not prevent it. For serious forms of the disease, it is best to combine penicillin with globulin. Administration of anthrax globulin combined with penicillin is the most effective method of treatment of cutaneous anthrax. However, V. N. Nikiforov stresses the fact that there are still cases of anthrax for which penicillin is ineffective, and this makes it necessary to search for new, more rational therapeutic agents. That author also indicates that it is imperative to bear in mind the possibility of long-term elimination of B. anthracis from the crust and feces after discharging anthrax patients.

As an illustration, we submit the case history of a patient who was under the observation of V. N. Nikiforov (1960).

> Patient M., 54 years old, was admitted in serious condition to the hospital on the 3d day of illness. Two or three days before his illness, he had removed the skin of a goat stricken with anthrax.
>
> Examination findings: carbuncle, 1×1 cm in size, on the left cheek, closer to the ear, with a black fundus, inflamed border and significant serous discharge. The entire right half of the face is edematous, the eye is closed. Submaxillary and lateral cervical lymph nodes on the left are enlarged, firm and slightly tender. The tongue is dry, coated with a thick brown film. Pulse 100, rhythmic, soft. Heart sounds are dull. Respiration is fast. The patient is dazed, has difficulty in answering questions. No changes were found in other organs.
>
> B. anthracis isolated from the discharge of the carbuncle on the day of admission. For the last 3 days, his condition continued to worsen in spite of pencillin and anthrax serum therapy. Edema successively involved the left side of the face at first, then extended to the cervical region and down to the clavicle (Figure 9). Diarrhea appeared on the first day of hospitalization, with concurrent moderate tenderness and distention of the abdomen. B. anthracis were isolated from excreta on the 9th and 11th days of illness. His condition began to improve on the 7th day of illness. The dazed state, tachycardia, dyspnea, tenderness and distention of the abdomen disappeared. Edema and diarrhea disappeared at almost the same time, by the 12th day. On the 18th day, the patient was discharged in satisfactory condition, with a crust at the site of the carbuncle and negative results of examination of excreta.

Other antibiotics have also been used in the treatment of anthrax. Some authors believe that antibiotics should be ranked in the following order, according to efficacy: biomycin, terramycin, levomycetin, penicillin, streptomycin, neomycin. Lincoln et al. (1964) recommend pencillin combined with streptomycin for sept forms of anthrax, while they leave chlorotetracycline and oxytetracycline at the choice of the physician. With regard to chloramphenicol, although we have not had experience with it ourselves, we can refer to the low efficacy reported by other researchers. Finally, the authors obtained the best results with the combination of antibiotics and serotherapy.

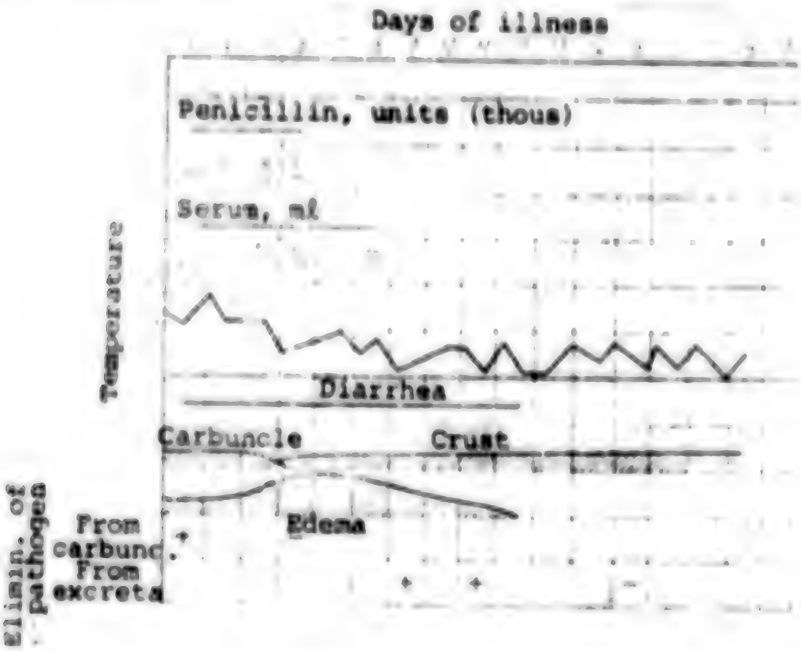


Figure 9. Extensive anthracic necrosis with crust in patient M. (after V. N. Nikiforov)

The dosage of these agents depends on the severity of clinical signs and improvement of the patients' condition. Biomycin is prescribed at first at the rate of 0.5 g every 4 h, and with improvement 0.5 g every 6 h; the average per course is 8 to 16 g. Terramycin is recommended in doses of up to 3 g (0.75 g 4 times) for the first 3-4 days, then 0.5 g every 6 h; the total for the course (6-8 days) is 16-18 g. Levomycetin is given at the rate of 1g 4 times, then 0.5 g 4 times. The course lasts 1-2 weeks. In this time, a patient receives 15 to 30 g of the product. Streptomycin is given intramuscularly in doses of 500,000 units twice a day until there is clinical improvement.

In view of the side-effects of antibiotics and intolerance thereof, as well as their efficacy only at the first stage of the disease, they should be used on a limited basis. In this regard, long-acting antibiotics (pencillin, levomycetin) may be promising, in the opinion of Lincoln et al. (1964).

With regard to pathogenetic agents, Lincoln et al. (1964) recommend oxygen therapy, administration of liquids and mineral salts (particularly calcium salts), and in the presence of shock and severe intoxication steroids and other hormones.

It is very important to regularly prescribe various symptomatic agents and particularly cardiac products. Much significance is attributed to meticulous patient care, with assignment of special medical personnel.

As for topical therapeutic measures, a dressing with some sort of ointment (boric, dermatol, zinc, etc.) is applied to accelerate healing of ulcers, carbuncle and pustules. One can also recommend dressings with pencillin or biomycin ointment.

In conclusion, it must be noted that antibiotics combined with serotherapy are the principal therapeutic agents for anthrax, and more recently specific anthracic globulin has been added. Pathogenetic agents, including steroids in the presence of signs of severe intoxication, are also important.

LABORATORY DIAGNOSTICS

Collection of samples for examination and conditions for working with anthracic material: The following are subject to laboratory examination for diagnostic purposes: serous discharge or tissular exudate from under the crust or bottom of the ulcer (if the cutaneous form is suspected), blood (if septic form is suspected), sputum (suspicion of pulmonary form), feces and urine (suspicion of intestinal form). From cadavers, the spleen, liver, lymph nodes, etc., are taken.

It is best to collect material for bacteriological examination before starting to treat the patient, since the use of immune serum (globulin) or antibiotics makes it difficult to isolate the pathogens of anthrax from the patient.

The contents of a vesicle or pustule, discharge from an ulcer or carbuncle are collected with a syringe or Pasteur pipette into a sterile tube or small glass vial. The material must be collected without injuring the tissue around the lesion.

When taking blood for bacteriological examination, two thick smears are prepared which are air-dried.

When indicated, sputum, feces and urine are collected in sterile containers.

The pathological material that is to be examined can be inoculated on nutrient media right at the patient's bed side, then sent to the laboratory.

When examining dead animals, the dissection of which is not recommended to avoid spreading infection, one cuts off the ear on the side on which the carcass was lying. First, the ear is ligated at the base, in two places, with tightly tied twine, and it is cut off between the ligatures. Without removing the twine, the ear is wrapped in a piece of cloth saturated with 3% phenol, then in wax paper. If the carcass was submitted to a post mortem, one takes parts of the edematous connective tissue, altered lymph nodes and pieces of spleen for examination.

The material to be examined, transported, stored, etc., is handled in accordance with the rules for working with particularly dangerous infections, which are spelled out in the appropriate instructions approved by the deputy USSR minister of health on 21 June 1967.

The accompanying label indicates the surname, name and patronymic of the patient, place and time of collecting the material, its name and tentative diagnosis. The collected material is put in a wood or metal box, which is tied up, sealed with lead or sealing wax. The following inscription is provided on the lid: "Top, handle with care," and it is sent by messenger to the laboratory. A paper is sent with the box of material, which indicates the objects to be examined and data listed on the labels of each test tube, glass, etc.

After the laboratory examination, the pathological material, infected animals and all waste from them must be submitted to autoclaving at 120° for 1.5 h or burned.

The instruments and syringes used for the examination are boiled in 1% soda solution for at least 1 h. Glassware is autoclaved or sterilized in 5% activated chloramine solution for 2 h. The surfaces of laboratory tables and other objects that could have been contaminated while working with B. anthracis are treated with 5% activated chloramine or cleared 20% calcium hypochlorite. Lab coats, cotton-gauze masks and caps [head scarves] are autoclaved. Rubberized aprons, sleeve protectors and rubber boots are treated with chloramine solution (R. A. Saltykov, 1967).

Examination methods: The main scheme for laboratory examination of pathological material is as follows: a) microscopy of stained smear; b) inoculation on nutrient media; c) biological test (infection of laboratory animals).

Microscopic examination of a smear, stained after fixation by the Gram method, permits visualization of large Gram-positive bacilli with blunt ends, often arranged as chains of different lengths, if B. anthracis is present.

Aged Loffler's methylene blue, Giemsa method or Rebiger's solution are used for demonstration of capsules. In the latter case, 15-20 g of gentian violet is dissolved in 100 mℓ ordinary formalin, the solution is allowed to stand at room temperature for a few hours and then filtered. This solution is used to stain unfixed smears for 15-20 s, then they are washed in water and dried. The capsules are stained a red-violet color and bacteria dark violet.

The presence in a smear of capsular form of bacilli justifies the tentative diagnosis of anthrax, since a capsule may also be present with other types of microbes. Upon demonstration of spores in the smear, one should consider another infection, since B. anthracis does not sporulate in anthrax patients or undissected cadavers.

Culturing on nutrient media has the objective of isolating a pure culture of the pathogen. The material obtained from the patient is inoculated, after preparation of smears for microscopy, on a dish with beef-extract agar and a tube with beef-extract broth or Hottinger's medium (pH 7.2). The morphology of B. anthracis growth on these nutrient media is one of its relative differential signs. It must be borne in mind that one does not succeed in culturing the pathogen in about 50% of the cases of cutaneous anthrax (Ye. I. Silant'yev, 1964).

After incubation for 24 h at 37° in beef-extract agar, a pure culture of the pathogen in the typical R form is obtained in the form of dull, rough colonies with shaggy margins, which were named "lion's mane" or "Medusa head" because of their typical appearance at low magnification. In addition to the typical rough colonies, there may be smooth, dwarf and intermediate colonies.

When cultivated in broth, a moderate sediment is formed at the bottom of the test tube in the form of small flocks, and the broth usually remains clear.

Several researchers consider it mandatory to also inoculate pathological material on blood agar to assess the hemolytic activity of the pathogen under study. B. anthracis does not show hemolysis during the first day of incubation.

The isolated cultures are identified on the basis of the signs that are the most typical of B. anthracis: smears are examined under a microscope to examine morphology of the microorganisms, they are examined in a hanging drop or semiliquid agar for demonstration of lack of motility. In doubtful cases, inoculation on gelatin is used to examine proteolytic capacity, tests are made with penicillin and anthrax bacteriophage.

When the culture under study is inoculated in a column of beef-extract gelatin, growth in the form of an upside down "fir tree" is observed along the inoculation site, which is inherent in B. anthracis. However, this phenomenon may not be present in some strains of B. anthracis.

The penicillin test ("pear necklace" test) is based on the fact that B. anthracis cells break down into spheres when incubated on beef-extract agar with penicillin. To run this test, one uses three dishes with 2% beef-extract agar, at the bottom of 2 of which one adds penicillin (0.5 units/mℓ in the first, 0.05 units/mℓ in the second), and 1 drop of 3-h broth culture of the tested strain is applied. One can run 10-15 tests with each dish. The cultures are put in the incubator for 3 h at 37°, then the dishes are examined under a microscope with a strong dry or immersion system (in the growth area the agar is covered with a cover glass).

On a medium containing penicillin, B. anthracis is demonstrable in the form of separate spheres--"pearls," arranged in the form of a chain, which is why this method was named the "pearlnecklace" test. In the control dish, the cells of B. anthracis retain their usual form, and they are arranged in chains of different lenths. In the opinion of E. N. Shlyakhov and Ye. V. Gruz (1967), the "pearl necklace" test is one of the most effective means of identifying B. anthracis.

The test with anthracic bacteriophage is based on demonstration of the phenomenon of lysis of an antrhax culture by specific bacteriophage. The sensitivity of the test culture to anthracic bacteriophage is checked by the dish method or by the "sliding drop" method.

When examined by the dish method, one drop of 3-h broth culture is put in each of 2 dishes with 2% beef-extract agar, Hottinger agar or blood agar. Anthracic bacteriophage in a test dilution is applied to one of the dishes, and the second is a control. The dishes are put in the incubator for 3-4 h at 37°, after which the action of phage is evaluated according to lysis of colonies (according to "Temporary MRTU-42 [interrepublic technical specifications] for Diagnostic Anthracic Bacteriophage").

For the "sliding drop" method, the tested culture is inoculated in 3-4 test tubes with slant beef-extract agar. After 20 min in the incubator at 37°, one drop of undiluted anthracic bacteriophage is applied to the test culture in 1-2 tubes, while 1-2 tubes serve as a control. One begins to assess the action of the phage after 6-9 h of incubation at 37°; however, better test results are obtained after 16-18 h. In the tubes with phage, lysis of the anthracic culture is observed along the path where it runs down, whereas there is ordinary growth around the zone of lysis. The usual growth of the culture is observed in the control tubes.

Biological tests (infection of laboratory animals) permit definitive identification of the tested culture as B. anthracis.

Animals are usually infected concurrently with making cultures on nutrient media.

Infection is performed with a 10% suspension of the tested material in sterile saline or sterile water. White mice are given 0.1-0.2 mℓ hypodermically in the bottom part of the back and guinea pigs, 0.4-0.5 mℓ hypodermically in the abdominal region. Other methods of infection are also used, for example, rubbing the tested material into a scarified area of skin.

The animals are kept under observation for up to 10 days, although the infected animals usually die within 1-3 days with signs of generalized sepsis if pathogenic microorganisms are present in the tested material. To speed up the answer, one can sacrifice a mouse 12 h after infection and submit to microscopic and bacteriological examination the tissue fluid near the site of injection of the material.

Dead animals are dissected, replica plates are prepared and cultures of blood from the heart, as well as spleen, liver, kidneys and infiltrate at the site of injection of infected material. In the presence of anthrax infection, the smears demonstrate capsular forms of B. anthracis and a typical form of culture growth on nutrient media.

Typical pathoanatomical findings on dissected animals are also a diagnostic criterion: at the site of injection of the infected material one generally observes a jelly-like hemorrhagic edema of subcutaneous tissue, hyperemia of the viscera and liquid, uncoagulated blood.

Definitive identification of the tested culture as a virulent form of B. anthracis is made by testing its virulence on rabbits. Infection of guinea pigs and mice for this purpose does not permit differentiation of the isolated culture from vaccine strains of B. anthracis.

One must prepare a spore culture on beef-extract agar, Hottinger's agar or on a medium made of casein broth (pH 7.2-7.4). The basis of this medium is a tryptic casein broth, to which one adds a diluted water extract of yeast and various salts (Gladstone-Field medium). The following is used to prepare 1 ℓ of medium: 50 mℓ tryptic casein broth, 200 mℓ water extract of yeast or 0.5% Bacto yeast solution, 5 g potassium diphosphate (K_2HPO_4), 0.01 g iron sulfate ($FeSO_4$), 30 g agar and distilled water to 1000 mℓ. One can also add 0.05 g magnesium sulfate ($MgSO_4$), 0.03g manganese sulfate ($MnSO_4$), 0.1 g calcium chloride ($CaCl_2$), 0.3 g sodium sulfate (Na_2SO_4) and 1 g potassium monophosphate (KH_2PO_4), dissolving them in the listed order after iron sulfate. Medium pH is brought to 7.2-7.4 with buffer and phosphate.

The culture to be examined is inoculated on this medium, and up to 90-95% spores are produced on the 3d-4th day in the incubator at 35-37°. The sporulation process is examined ["checked"] by examining smears stained according to Ziehl-Neelsen. Then the spore culture is washed off the agar with distilled water and imbedded in 50% glycerin.

Presence of virulence can be determined by giving 1-2 rabbits 10,000 or 100,000 spores each. To determine the degree of virulence, 3 doses of spore culture, 100, 1000 and 10,000 spores in a volume of 1 ml, are given to rabbits weighing 2-2.5 kg in the abdominal region (to obtain these doses, the spore culture is diluted in 0.15 M NaCl solution to GKI [State Control Institute of Medical Biological Products] cloudiness standard No 10, which corresponds to about 100 million anthrax spores, and the required doses are obtained from subsequent dilutions). Each dose is given to two rabbits. In all, six rabbits are needed to determine the degree of virulence of one culture.

The animals are kept under observation for 10 days. Smears are prepared from the organs of dead animals for bacterioscopy and culturing on appropriate nutrient media to identify the isolated culture. All of the studies dealing with isolation and identification of anthrax cultures are conducted in accordance with the specifications for working with the pathogens of particularly dangerous infections.

DETECTION OF PATHOGEN IN THE ENVIRONMENT

Collection of samples: B. anthracis may be present in raw material of animal origin (wool, pelts, hair, etc.), by-products and food products recovered from anthrax-stricken animals, as well as objects contaminated with it, in soil of areas where anthrax-stricken animals are maintained, where they died, were cut up and buried, in the water and silt of open reservoirs, in the dust and air of rooms, places where animal raw material is collected, stored, transported and processed, in blood-sucking arthropods which are the vectors of anthrax infection, etc. For this reason, meat, pelts, wool, fodder, soil, water, air, etc., may be submitted to examination.

When examining contaminated soil regions, a circumscribed area is identified, and it is broken down into blocks. Five points are designated in each block in a diagonal line, or four points along the edges and one in the middle, from which one then collects soil samples. In pastures and areas where animals are maintained, samples are taken at a depth of 5-20 cm using a metal scoop or light-weight drill. Soil samples are taken from places were carcasses of anthrax-stricken animals are buried from different layers to a depth of 1.5-2 m. Each sample weights 30-40 g.

Water samples are taken in amounts of 1-2 ℓ. In addition, when examining open reservoirs, analysis is made of samples from the surface layer of silt at the bottom of the reservoir in amounts of about 100 g.

Various methods of trapping and concentrating aerosol using the instruments of D'yakov, Krotkov, the bacterial trap of Rechmenskiy, diaphragm filters, etc., are used to demonstrate B. anthracis in air.

When examining blood-sucking arthropods, they are cut up and ground, whereas for special analysis one takes the proboscis, legs and ground gastrointestinal tract.

Examination methods: The methods for demonstration of B. anthracis on different environmental objects are divided into informative (rapid) and conclusive methods. The informative ["signal"] methods make it possible to gain a tentative idea about the presence of B. anthracis in the material examined, and they must be followed by mandatory conclusive examinations, which are decisive.

The instructions dealing with examination of water, soil, washings from environmental objects for demonstration of B. anthracis, which were approved by the USSR Ministry of Health on 12 June 1965, refer to the following informative methods: luminescence-serological method, method for demonstration of capsule formation on GKI medium, method for demonstration of capsule formation in mice.* These methods are interchangeable, depending on specific conditions; however, the mandatory requirement is that at least two of them must be used at one time.

The conclusive methods of examination, which are aimed at isolation of pure culture and examination of its properties, include the following: examination of motility and morphology of the culture, running test with pencillin, determining sensitivity to specific bacteriophage, gelatin test and determination of virulence of the culture in rabbits. The methods for conclusive examinations to identify cultures of B. anthracis are described in the section entitled "Laboratory Diagnostics."

To prepare for analysis of soil, a batch of 30 g is covered with 100 mℓ water, agitated for 5-10 min, after which the suspension is allowed to stand for 1-2 h. The supernatant is decanted and brought to 1 ℓ with sterile saline. The preliminary preparations are the same for silt.

It is recommended to use amounts of 1 ℓ for examination of washings from environmental objects or extracts from wool, skin, etc. Water for analysis is also taken in amounts of 1 ℓ. In the presence of marked opalescence or suspended particles in the fluid under examination, it should first be filtered through sterile gauze, cotton or coarse paper filters, which can then be also submitted to examination.

Water or supernatant fluid to be examined is separated into two equal parts (500 mℓ each), one of which is heated to 65-70° for 30 min. In order to concentrate the microorganisms, unheated fluid is passed separately through four No 3 diaphragm [or membrane] filters in equal batches (if there is not enough of the material to be studied, one can pass 10 mℓ through each filter). Two filters are inoculated on dishes with 0.7% beef-extract agar by placing the filters on the nutrient medium for 3 h, and they are used for luminescence-serological analysis. A washing is prepared from the rest of the filters in sterile Petri dishes using 1 mℓ 0.15 M NaCl solution, and it is used for inoculation on GKI medium, as well as administration to white mice. Similar manipulations are performed with the heated liquid.

The luminescence-serological method is based on demonstration of B. anthracis antigens by means of specific sera combined with fluorescent dyes (Ye. N. Levina). The sensitivity of this method is extremely high; however, it is considered suitable only for demonstration of vegetative forms of B. anthracis.

The material to be examined is first incubated at 37° for 3 h, then a washing is prepared from each membrane filter with 0.15 M NaCl; smears are prepared from the obtained suspension, they are dried and fixed in 96° methanol for 10-15 min. The preparations are then treated with special luminescent sera in dilutions that produce bright fluorescence of B. anthracis, and they are examined under an ML-1 or ML-2 fluorescence microscopy or ON-17 luminescence device with a system of blue or violet filters. Examination is made at magnifications of 5×90, 7×90 and 10×90 .

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*Informative methods based on demonstration of capsule formation are not suitable in the rare cases when acapsulogenic strains of B. anthracis are present on environmental objects; for this reason S. N. Shlyakhov and Ye. V. Gruz (1967) propose that a test for lysability with specific bacteriophage be included in the group of informative tests.

The degree of fluorescence of microbial cells is indicated by a different number of crosses: ++++--very bright fluorescence of cell periphery with clear contrast with the dark body of the cell; +++--bright fluorescence of cell periphery; ++--mild fluorescence of cell periphery, resulting in negligible contrast to its body; + and ±--very mild fluorescence of cell outline.

Demonstration against the dark background of the preparations of green, bright fluorescence on the periphery of microbial cells (with fluorescence graded at ++ or +++) with the typical morphology of B. anthracis should lead one to suspect that it is present in the sample examined, and justifies a tentative, informative positive answer.

The isolated culture must then be submitted to complete identification.

Demonstration of capsule formation in vitro (method of GKI, Arkhipova) is based on the capacity of B. anthracis to form capsules on the special GKI nutrient medium.

GKI (State Control Institute of Medical Biological Products) medium is prepared as follows: 60 mℓ sterile Hanks' solution is placed in a sterile flask and 40 mℓ ox serum is added (under sterile conditions) after inactivating the latter at 56° for 30 min. After thorough mixing, the pH of the medium is brought up to 7.2 with soda solution and it is decanted at the rate of 2 mℓ per tube. The tubes are closed with sterile rubber stoppers. The medium can be stored for a long time in this form at a temperature of 4°.

The suspension under study or washings from filters that served to concentrate bacteria are inoculated on GKI medium and put in the incubator for 37° in order to test the culture's ability to produce capsules. It is believed that most B. anthracis in the material understudy form a capsule on GKI medium after 16-18 h. Smears of this medium are fixed with methanol for 15 min, stained with Loffler's blue for 5-10 min and examined under a microscope at magnification of 900×. The capsule is stained pink and the microbial bodies blue.

Demonstration of encapsulated cells in the preparations with GKI medium justifies a tentative positive answer with respect to B. anthracis, while the obtained culture must be submitted to further examination.

In the absence of Hanks' solution, one can obtain satisfactory results concerning capsule formation by culturing the material under study in Hottinger's broth with addition of 40% inactivated ox serum (R. A. Saltykov).

The method of demonstration of capsule formation on selective medium was proposed by the Bulgarian authors, A. Tomov, T. Todorov and I. Benvenisti (1965). The formula for this medium is as follows: 50 mℓ peptone agar (10% agar, 2.5% peptone, 0.5% sodium chloride), 100 mℓ bovine serum, 50 mℓ egg albumin, 25 mℓ 0.4% hemin in 100 N caustic soda, 25 mℓ 100 N acetic acid; the medium pH should be exactly 7.2. This medium depresses development of most extraneous sporulating bacilli. The cultures are put in an incubator for 15-18 h at 37°. Smears of this medium are treated with methyl alcohol or formal-gentian violet, as well as fluorescent sera (in the latter case, they are examined under a fluorescence microscope).

The method of demonstrating capsule formation in vivo (method of Shlyakhov and Gruz) is based on the capacity of B. anthracis to form a capsule in a living organism.

When using this method, 0.1-0.2 mℓ washings from diaphragm filters are given intramuscularly to 8 white mice (if the necessary number of animals is unavailable, only 2 are infected), then 2 mice at a time are sacrificed 30, 60 and 120 min after the injection. Two mice are left as a control, and they are not sacrificed until the end of the examination. The mice are dissected, smears and replica plates, which are examined for capsular microbes, are prepared from the peritoneal exudate and organs. Positive results justify an informative [signal, warning] answer and continuation of tests in order to identify the culture.

The final conclusion is derived on the basis of a number of signs inherent in B. anthracis, which permit differentiation thereof from other Gram-positive, sporulating aerobic microorganisms that could be present on various environmental objects (Tables 6 and 7). Some differential signs of B. anthracis are relative, while others are obligatory. The relative signs include the typical morphology of growth on solid and liquid nutrient media and sensitivity to penicillin; obligatory ones include lack of motility, capsule formation, fluorescence under the influence of specific fluorescent sera, sensitivity to specific bacteriophage and pathogenisity for laboratory animals (pathogenicity for rabbits for virulent cultures) (N. N. Ginsburg, 1966).

Ascoli's precipitation reaction: A method of serological diagnostics of anthrax, which is called the precipitation reaction or thermoprecipitation according to Ascoli, is very popular. This reaction demonstrates specific anthracic antigen, precipitinogen, in the material under study; this antigen reacts with precipitating serum obtained from horses that have been immunized in accordance with a special system. The Ascoli reaction is convenient in that it yields reliable positive results in cases where bacteriological tests do not permit demonstration of B. anthracis, for example, in the case of examining decomposing material that is not fresh. This reaction is used to analyze the organs of a dead animal, to examine wet pelts, in the tanning and wool-processing industry.

The following are required for the precipitation reaction: precipitating anthracic serum, antigen extract from the material under study and anthracic bacterial antigen for the control.

To obtain antigen-extract, pieces of skin, wool, muscle, etc., are ground, covered with saline at the rate of 10 mℓ solution per gram material to be examined, followed by extraction by boiling for 10 min. The extract is filtered until it is entirely clear through calcined asbestos cotton, a talcum filter or paper saturated with saline.

There is also a cold method of preparing antigen, which is considered more reliable and can be used to check the thermoprecipitation reaction. The material can first be sterilized in an autoclave. Then it is ground, covered with 0.85% sodium chloride and 0.3% crystalline phenol solution in a ratio of 1:10, and allowed to stand for 20 h at room temperature or 30-40 h at 4-10°. The obtained extract is filtered through an asbestos filter, which has a neutral reaction to litmus, until it is entirely clear (Ye. I. Silant'yev, 1964).

Table 6. Differential signs of B. anthracis and related sporulating aerobes (after Bergi, 1957)

Species	Morphology	Motility	Capsule formation	Growth on gelatin	Growth on broth	Acetylmethylcarbinol product.	Lecithinase prod	Pathogenicity
B.anthracis	Rods 1-1.3×3-10	Non-motile	+	Slow dilution, upside down fir tree type	Growth at bottom of tube in a floccule, there may be a friable ring near wall, slight cloudiness	Yes	Yes	To man, cattle, pigs, sheep, rabbits, guinea pigs and mice
B. cereus	Rods 1-1.2×3-5	Motile	−	Rapid dilution	Uniform cloudiness during growth with sediment, with or without film	Yes	Yes	Large doses of 24-h culture are deadly to guinea pigs
Variants	0.8-1.3×2-6	Non-motile	+	Same				
B. cereus var. micoides	Very fine rods Note: Other features similar to B.cereus							
B. megatherium	Rods 1.2-1.5×2-4	Motile	−	Slow dilution	Uniform cloudiness during growth with or without sediment, no film	No	No	
Variants	0.9-2.2×1-5	Non-motile						

Table 7. Differential morphological and biological features of B. anthracis and

Name of bacillus	Motility	Reaction to oxygen	Sporulation	Capsule formation	Gram staining	Liquid media	
						beef broth	broth with blood
B.anthracis	-	-	-	+	+	Growth after 7-8 h. After 18-24 h broth remains transparent with floccules, readily scattered sediment; no film is formed. If ring is formed near wall it can be readily washed off	No hemolysis
B. pseudoanthracis	Mild +	-	-	-	+	Broth becomes cloudy, crumb-like sediment forms at bottom, which is difficult to break up. Ring on wall that is difficult to wash off, film forms on surface	Hemolysis
B. anthracoides	Mild + Motility stops when sporul. begins	+	+	-	+	Same	Hemolysis
B.subtilis	Active +	+	+	-	+	At first turns cloudy, then becomes clear after formation of film	-
B. mesentericus	Active +	+	+	-	+	Remains virtually clear with formation of wrinkled film	-
B. megatherium	Mild +	+	+	-	+	Negligible sediment without formation of film	-
B.mycoides	Mild +	+	+	-	+	Broth remains clear. Cotton-like clump at the bottom which does not break up when agitated	-

One decants 0.2-0.3 mℓ clear anthracic serum into narrow test tubes, then carefully superimposes the antigen under study carefully (along the wall) in the same amount.

When the reaction is positive, there should appear a dull white precipitation ring at the boundary between the two liquids no later than after 15 min. This reaction is graded as ++. When the reaction is questionable, the ring appears after more than 15 min, and the result is graded as ± (when examining skin, if a doubtful reaction is obtained twice it is deemed positive). A negative result is graded with a -.

Control tests are run concurrently: 1) the precipitating serum and extract from material known to be anthracic (positive reaction); 2) precipitating serum and extract from the organ of an animal known to be healthy (negative reaction);

other soil bacilli

Solid media		Pathogenicity for		
gelatin	agar	mice	guinea pigs	rabbits
Horizontal processes give off from whitish shaft of stab line. Findings resemble upside down "fir." Stocking-shaped dilution after 3-4 days	White, fluffy, young colonies, consisting of curl-like twists of filaments at low magnification	Death within 24 h	Death within 24-36 h	Death within 36-72 h
From the shaft along stab line many whitish branches. Gelatin is markedly liquefied	Firm, round, whitish colonies, tortuous filaments along edges that fall apart	Sometimes pathogenic for mice when large amounts of culture are injected into abdominal cavity	Apathogenic	Apathogenic
Nodular thickening along stab line, but no processes. Gelatin dissolves in the shape of a funnel	Same	Same	Same	Same
Colony surrounded by ring of rays. Dissolved gelatin film on surface	Gray-white dull coat rising on tube wall. Film in condens. water	Apathogenic	Same	Same
Round colonies with dissolution of gelatin	Thick, wrinkled dull coat with film in condensation water	Same	Same	Same
Gelatin is dissolved in the shape of funnel	Gray, mucous, white film	Same	Same	Same
Gelatin dissolves rapidly	Delicate fibrous superpositions	Same	Same	Same

3) precipitating serum and saline or distilled water (negative reaction); 4) normal serum and the extract under study (negative reaction).

PREVENTION AND CONTROL MEASURES

The measures for the prevention of anthrax are implemented in accordance with the specific complex plans for the control of anthrax in a given republic, oblast (kray), rayon, etc.

The plan is prepared jointly by the concerned agencies and institutions, and it is approved by the council of ministers of Union and autonomous republics, ispolkoms of councils of workers' deputies.

The set of veterinary-sanitary anthrax control measures implemented in the Soviet Union in accordance with the "Instructions on Measures Against Anthrax," approved by the USSR Ministry of Agriculture on 28 February 1953, includes the following:

1) detection, record and mapping of areas that have permanent anthrax problems
2) complete coverage of farm animals with immunization in problem areas
3) ameliorative and agrotechnical measures to disinfect problematic areas and bodies of water
4) supervision of proper condition of cattle burial grounds, cattle-driving routes, pastures, livestock buildings, etc.
5) supervision of adherence to veterinary and sanitary rules in procurement, storage, transportation and processing of raw material of animal origin
6) early identification of anthrax among animals, isolation and treatment of sick animals, epizootiological inspection of disease site, decontamination of carcasses of animals that died of anthrax, routine and final disinfection of anthrax site
7) veterinary-sanitary educational work among the public, and others.

The set of anthrax control measures, which have been specified for health agencies in the "Instructions for Clinical and Laboratory Diagnostics, Treatment and Prevention of Anthrax in man," stipulates the following:

1) implementation of general sanitary preventive measures: a) among the public in areas that are permanently problematic for anthrax, particularly livestock farms and among private cattle owners; b) among individuals who work at enterprises dealing with procurement, storage and processing of raw material of animal origin
2) preventive inoculations for individuals exposed to the risk of anthrax infection
3) detection, recording, epidemiological inspection and decontamination of anthrax sites
4) mandatory hospitalization and treatment of victims of anthrax
5) sanitary educational work among the public, etc.

Preventive inoculations against anthrax for people: Live, acapsular STI sporulated vaccine is used to inoculate man against anthrax.

The vaccine is highly effective and mildly reactogenic.

Inoculations are indicated for individuals who have been exposed to the risk of infection: 1) zootechnical and veterinary workers; 2) workers at slaughtering houses and meat-packing plants; 3) individuals engaged in procurement, sorting, storage, transporation and processing of raw material of animal origin (pelts, hides, wool, bones, bone meal, etc.); 4) individuals who come in contact with public or privately owned farm animals in areas where there is an anthrax problem; 5) individuals working with live anthrax cultures, laboratory animals infected with anthrax or who examine material infected with anthrax; 6) when there are epidemiological indications.

Inoculations are contraindicated in the presence of acute infectious disease or exacerbation of chronic diseases, febrile state, marked insufficiency of the cardiovascular system (heart diseases at the decompensation stage, essential hypertension grade III, coronary insufficiency, etc.), cachectic state, diabetes, serious acute and chronic diseases of internal organs (exacerbation of peptic ulcer, acute nephritis, acute lesion and cirrhosis of the liver, bronchial asthma, marked pulmonary emphysema and others), thyroid diseases (second-third grade), lymphogranulomatosis, malignant neoplasms, dermatological diseases with extensive lesions to the skin surface.

The inoculation is performed by a physician or specially trained paramedical personnel. Vaccination is performed once and repeated annually.

The vaccine must be stored in a dry, dark and cool place at a temperature of 4° (storage at below 0° is permitted). The shelf life of the vaccine is 2 years from the day it was manufactured. Lots of vaccine that have not been used up within 2 years (when at least 5000 doses remain) must be rechecked by the institution that produced it in order to determine whether shelf life can be extended.

Before administering the inoculation, the dry vaccine is diluted under sterile conditions in 1 mℓ sterile aqueous solution of 30% glycerin, which is furnished in a separate vial with the vaccine. The obtained bacterial suspension is used for vaccination.

The vial and its contents are thoroughly inspected before and after diluting the vaccine. If there are cracks in the vial, extraneous admixtures in the vaccine, undissolved floccules and nonuniform suspension, the vial is discarded and destroyed.

The vaccine is used within 4 h after the vial is opened. Inoculations are administered epicutaneously, using a smallpox vaccination needle, through the scarified skin of the external aspect of the middle third of the arm. The skin is first rubbed with alcohol (iodine, sublimate and other disinfectants must not be used!), then with ether to remove fat. Using a sterile tuberculin syringe with a thin needle, one drop of vaccine is applied to the two sites of the future inoculation, at a distance of 3-4 cm, without touching the skin. The material should spread on the skin evenly, in the form of a spot. Using the smallpox vaccine quill, 4-5 scratches (not cuts!) are made through the layer of applied vaccine. The scratches must be superficial and should not bleed. After this, the vaccine is rubbed into the scratches for 30 s using the flat surface of the same instrument. One must allow 5-10 min for the vaccine to dry after the inoculation.

Usually no general reaction is observed in inoculated subjects. The local reaction consists of slight reddening of the scratches, with formation of small crusts after 2-3 days, which soon (on the 4th-8th day) fall off.

A check of whether the vaccine has taken is made after 48, 72 and 96 h, as well as on the 8th day after vaccination or revaccination. The local skin reactions are graded with a + when the result is positive and a - when it is negative. Presence of marked redness and swelling on the skin along the abrasions is considered a positive result, and absence of skin reactions, a negative result.

The inoculated individuals are placed under medical supervision in accordance with the general rules. The inoculated individuals, as well as results of postinoculation examination are registered in a log, which must contain the surname, name, patronymic, age, occupation, home address, date of vaccination, lot and shelf life of vaccine, date of recheck and result of inoculation on the 8th day, nature of local and systemic reactions, surname and position of individual who gave the inoculation.

Mandatory measures when people contract anthrax: The following mandatory measures are implemented when a case of anthrax appears:

1) report about the case
2) hospitalization of anthrax-stricken patient or one suspected of suffering from this disease
3) final disinfection at the site
4) epidemiological inspection
5) immediate (emergency) specific immunization of individuals exposed to the risk of infection.

When there is a case of anthrax, this is reported by telephone to the superior medical institution, with simultaneous mailing of urgent notification and unscheduled report to the ministry of health of the Union republic, district veterinary worker servicing the populated center, farm or enterprise where the individual was infected, executive committee of the local council of workers' deputies.

All patients with anthrax (regardless of the form of the disease) or suspected of having this disease must be immediately hospitalized.

The patients are hospitalized in the infectious departments of hospitals, and if there are none, in separate wards.

Separate medical personnel is assigned to care for patients with pulmonary, intestinal and septic anthrax. All procedures at the patient's bed side are performed wearing rubber gloves and specially issued coats. It is mandatory to use protective glasses and a cotton-gauze mask when caring for patients with pulmonary and septic forms of the disease.

In the case of cutaneous anthrax, recovered patients are discharged after the scabs have fallen off, epithelization and cicatrization of ulcers; after septic, pulmonary and intestinal anthrax, they are discharged after clinical recovery and two-fold negative results of bacteriological tests performed at an interval of 5 days.

In the case of death of an anthrax-stricken patient, the cadaver is wrapped in a sheet soaked in 20% milky solution of calcium hypochlorite [calcinated lime], put in a coffin made of metal or closely nailed boards lined with oilcloth on the inside, on a layer of calcium hypochlorite 10 cm in thickness, and dusted with calcium hypochlorite on all sides. The lid of the coffin is nailed down and not opened again. When the necessary conditions are available, the coffin with the cadaver is burned. If cremation is not feasible, it is interred.

The corpses can be buried at an ordinary cemetery. The coffin is placed in the grave at a depth of at least 2 m. In areas where there is subterranean water at a high level, the coffin is placed in a sturdy box that is covered with tar inside and outside. The space between the coffin and the box is filled with dry calcium hypochlorite, the solid lid, which is also tarred, is nailed down tightly on the box.

If the diagnosis of anthrax has been confirmed by bacteriological tests, autopsies are not performed on victims of anthrax. If there is an urgent need for an autopsy on victims of anthrax, it is performed only by a physician, and followed by mandatory final disinfection of the room, all objects and instruments that were used, coats, gloves, shoes, etc.

The apartment of an anthrax patient, or individual suspected of having anthrax, is submitted to thorough final disinfection of the rooms, furniture, objects used in patient care, bedding, linens, etc. Disinfection is performed by employees of disinfection or sanitary epidemiological stations under the supervision and in the presence of a physician.

Everyone working at an anthrax site must be vaccinated against anthrax and provided with work clothes made of heavy [tightly woven] material (coveralls, rubber gloves, rubber boots).

The disinfection team works in gas masks, since chlorine and formaldehyde are toxic, hot solutions of caustic soda may cause ulcerations if they get on the skin and could lead to loss of consciousness if they get in the eyes.

Table 8 lists the methods of decontaminating objects and material infected with B. anthracis spores.

Clothing and work clothes of individuals who have come in contact with material that was or is suspected of being infected with B. anthracis are disinfected in the following manner: a) treatment in flowing steam chambers at 110° for 90 min (if this does not destroy the clothing); b) boiling in laundry boilers for 60 min with 1-2% soda solution; c) soaking in 1% activated chloramine solution for 2 h at a temperature of 20° using 5 ℓ disinfectant per kg clothing.

Rubber boots and rubber gloves are decontaminated in 4% formaldehyde solution for 4 h or 1% activated chloramine solution for 2 h.

A thorough epidemiological inspection is performed to determine the source and causes of infection. Special attention is given to the patient's occupation, nature of his work, where he was 7-8 days before the illness, etc.

Table 8. Decontamination of objects and material contaminated with B. anthracis spores

Objects and material to be disinfected	Routine disinfection	Final disinfection
Dressing material (cotton, compress paper, bandages, tampons, sponges, etc.), solid left-overs of patient's food, trash and nonvaluables	Collected after use into special container and immediately destroyed by burning or boiling in 2% soda for 60 min	Same as for routine disinfection
Liquid food left-overs	Boiled for 60 min in covered pan, after which they are flushed into sewer and, if none available into outhouse or drain pit	Same
Patient's dishes	Dishes used for drinking and eating are boiled after each use in 2% soda for 60 min, then rinsed in clean water	Same
Patient's dirty laundry and linen, coats and outer clothing of people around patient and service personnel	Decontaminated locally, soaking in 1% laundry soda then slowly brought to boiling point and boiled for 60 min. 1% activated chloramine or preparation KhB is suitable for decontamination, with 2-h exposure, as well as 0.2% formaldehyde mixed with 0.2% soap or 0.2% OP-10 solution at 60° for 60 min, 3% hydrogen peroxide with 0.5% Progress detergent at 50° for 40 min. The laundry is soaked in these solutions used in amounts of 5 ℓ per kg laundry. If disinfection by these methods is not feasible locally, the laundry is sent in a box to a disinfection center for decontamination in a steam or steam and formalin chamber	
Patient's bedding (pillows, mattress, cotton and wool blankets, bedside throw rugs, etc.)	Bedding is packed in boxes and sent to steam chamber for disinfection at 110-111° for 40 min, or steam-formalin chamber for disinfection at 62-63° for 2.5 h	Same
Patient's clothing worn 2-3 weeks before illness and at its onset, as well as all other objects that came in contact with such clothing or the patient	Packed in boxes and sent for decontamination in chamber by the steam-formalin (250 mℓ formalin per m^3 chamber, 58-59°, 2.5 h) or air-steam (97-98°, 45 min) method	Same
Room occupied by patient, as well as objects in it	Floor, walls, doors and other surfaces and objects are decontaminated 2-3 times a day by spraying with one of the following solutions:	Same

Table 8. (continued)

Objects and material to be disinfected	Routine disinfection	Final disinfection
	a) 4% clarified activated chlorinated lime containing 10 g active chlorine per liter b) 4% activated chloramine or KhB (activator--ammonia chloride or sulfate in a dosage of 5-10 g per liter) c) 20% clarified chlorinated lime or 15% calcium hypochlorite with at least 5% active chlorine d) 6% hydrogen peroxide with 0.5% Progress detergent e) hot (60°) 5% formaldehyde with 5% soap solution f) in rooms with walls painted with oil paint, one should use hot (55°) 5% formaldehyde with 5% soap, spraying them twice g) furniture and other objects in the patient's room to be thoroughly wiped with rags soaked in one of the above solutions. The used rags must be burned or decontaminated by boiling for 1 h in a covered pan Note : 1. Dirty apartments or rooms where products (meat, milk, etc.) or raw material (pelts, wool, etc.) from anthrax-stricken animals were handled are disinfected in the same way 2. Spraying is done at 30-min intervals, and disinfection is considered complete 30 min after the last spraying. It is recommended to disinfect rooms with the windows open 3. Outlay of disinfectants should be 900 mℓ for porous surfaces (brick and plaster) and 500 mℓ for nonporous ones per m^2 treated surface; when using hydrogen peroxide with detergents, 300 mℓ/m^2 surface	
Patient's excreta (feces, urine, sputum, vomitus)	Put with dry chlorinated line or ditribasic [?] calcium hypochloride in a ratio of 1:2 in relation to excreta (1:10 or 1:20 for urine) and mixed thoroughly. Excreta are left in this form for 2 h in covered container, then flushed into toilet or outhouse Note: The containers for collection of excreta must be enameled or of glazed pottery with a firmly closing lid.	Same as for routine disinfection
Chamber pots, urinals, bed pans, trays for expectoration	Contents are removed, then items are placed in a special tank with a good cover containing clarified 20% chlorinated lime for 60 min or 6% hydrogen peroxide with 0.5% Progress detergent at 60° for 6 min, then washed thoroughly in hot water. Separate containers for collection of excreta are assigned for each patient	

Table 8. (end)

Objects and material to be disinfected	Routine disinfection	Final disinfection
Outhouses, trash boxes, drain pits	Irrigated profusely with 20% milky solution of calcinated lime twice, at an interval of 3 h. Contents covered with dry calcinated lime to prevent fly invasion. The trash and inexpensive cleaning materials (rags, brooms, etc.) remaining after disinfection are burned	Same as for routine disinfection

It is imperative to make use of findings of veterinary-sanitary surveillance, clinical diagnosis (form of the disease) data on localization of lesion (with the cutaneous form), as well as results of laboratory tests on various objects, in order to determine the source and causes of the disease.

Anthracic globulin is used for emergency prevention of anthrax. The globulin is given for prophylactic purposes to individuals who have cared for anthrax-stricken animals, were involved in forced slaughtering, cutting of carcasses or burying stricken animals, who prepared food from the meat of sick animals or consumed such food. In some cases, globulin is given to individuals who have been around an anthrax-stricken patient, when there was particularly close communication during illness, contact with his bed, clothing.

Globulin must be used as soon as possible after contact with infected material. It is not expedient to administer globulin if more than 10 days have elapsed from the time of possible infection of the integument or more than 5 days after consuming meat from anthrax-stricken animals.

The recommended dosage for healthy adults is 20-25 mℓ globulin. The dosage for adolescents, 14-17 years of age, is 12 mℓ, and for children it is 5-8 mℓ, depending on their age.

Globulin is injected intramuscularly, mostly in the gluteal region. First, the individual's individual sensitivity to equine protein is checked by means of the intracutaneous test.

Globulin should be used in heated form, for which purpose the vial with the solution is placed in warm water (no higher than 40° temperature) for 10-15 min. A significant sediment at the bottom of the vial, strong cloudiness of globulin, film formation and a putrid odor are indicative of unfitness of the globulin, and it is forbidden to use such globulin.

Anthrax globulin used for emergency prevention of anthrax is given in combination with penicillin (500,000-1,000,000 units) and it is followed by STI vaccine inoculation 7-10 days later.

Globulin should be used in strict conformity with the "Instructions for Use of Anthrax Globulin," approved by the deputy USSR minister of health on 24 June 1965.

Prevention of anthrax in permanently problematic areas: According to the definition of L. V. Gromashevskiy (1965), a place where there has been an anthrax-stricken animal or person and the territory around it within the range, in which it is capable of infecting others in the vicinity, is called an anthrax site. In view of the fact that B. anthracis is capable of surviving for a long time in the environment, the following may serve as infection sites: places where livestock is maintained and slaughtered, where carcasses of anthrax-stricken animals are cut up and buried, where raw material, semi-finished or finished products infected with B. anthracis spores are stored or processed, where people who have been stricken with anthrax have been, etc.

The presently adopted definition of the concept of "permanently problematic area for anthrax" was formulated in paragram 3 of the instructions of the USSR Ministry of Agriculture dated 28 February 1953: "Measures to Control Anthrax": "Separate populated centers, sections of pastures and grazing grounds where cases of anthrax have been recorded among animals, regardless of how much time has elapsed since their occurrence, are classified as being permanently problematic for anthrax." However, it must be noted that the combination in this definition, as being equivalent, of the concepts of "separate populated centers" and "separate sections of pastures and grazing ground," should make it difficult, in our opinion, to keep a record of permanently problematic sites, since there may be several problematic sections of pastures and grazing grounds attached to a populated center. In this sense, it would be unclear whether this populated center should be registered as one or several permanently problematic areas. Evidently, it would be more correct to consider different sections of pastures and grazing grounds with an anthrax problem merely as soil sites of infection of a given permanently problematic area for anthrax. In this case, it could be considered that permanently problematic areas for anthrax will refer to separate populated centers with the lands belonging to them, where cases of anthrax have been recorded, regardless of how much time has elapsed since they occurred.

Determination of the epizootic status of problem areas is made by examining the migration of cases of anthrax among animals in prior years, regardless of how long ago, and examining the present epizootic state of a given center.

For this purpose, the veterinary administrations of the ministry of agriculture of the republic involved, veterinary departments of kray and oblast administrations of agriculture, with the participation of specialists from oblast veterinary laboratories, veterinarians of rayon agriculture departments and zooveterinarian districts must determine the following with regard to each location: 1) date and number of cases of anthrax for each animal species on the basis of statistics and other data; 2) presence of livestock burial grounds and separate anthracic graves, with a description of their location and condition at this time; 3) sections of pastures, grazing grounds, bases, yards and water reservoirs that have an anthrax problem; 4) topographic, soil and other local features of areas with anthrax-stricken livestock, how livestock is watered during the grazing period and the route followed by cattle to pastures; 5) location and veterinary-sanitary condition of meat-packing plants, slaughterhouses, salvage [heat recovery?] areas, warehouses and places where raw material of animal origin is processed, livestock receiving bases and vehicles used to transport livestock to the bases.

The veterinarian of a district (farm) furnishes a comprehensive epizootiological report for each site in a special log, on the basis of the above data. The chief veterinarian of the rayon department of agriculture plots an epizootic map of the rayon for anthrax on the basis of obtained information, indicating on it all of the centers with an anthrax problem, dates and number of cases of the disease and animal deaths.

It is imperative to include in the general plan of kolkhoz and sovkhoz land management ameliorative and agrotechnical measures aimed at sanitizing centers with an anthrax problem: draining swamps, swampy pastures and drying haylage, improving reservoirs and wells, etc. The sections with an anthrax problem must be excluded from farm use, and they must be marked off with noticeable signs. Livestock burial grounds must be fenced off to prevent access of animals and people.

In accordance with the "Sanitary Rules for Rural Planning, Construction and Utilities," approved by the USSR Main State Sanitary Inspectorate on 26 January 1956, it is forbidden to build populated centers and farm buildings in areas where anthrax-stricken animals have been buried in the past.

When preparing reservoirs or canals to be filled with water, it is imperative to reinforce burial grounds for anthrax-stricken animals situated in the zone of operation of the reservoir and 2 m below it. If the reinforcement cannot prevent erosion of these burial grounds, they can be moved. Livestock burial grounds situated in a coastal landslide area must also be moved.

Farm animals are immunized for preventive purposes in centers that have a permanent anthrax problem or that are threatened by it. The plans for the preventive inoculations are prepared by the chief veterinarian of the rayon department of agriculture, on the basis of data describing each problem area separately.

Preventive inoculations are given once a year to all susceptible animals over 2-3 months old (depending on the type of vaccine). The animals become immune 10 days after the inoculation and immunity persists for up to 12 months.

The inoculations are given only by veterinarians or experienced veterinary feldshers under the supervision of the veterinarian. An exact record must be kept of immunized animals, with mandatory record of results (reaction, complications). Inoculated animals are kept under veterinary supervision until the end of the reaction to the immunization.

The transfer of inoculated animals to other farms or for slaughtering is not allowed during the period that they are under veterinary supervision. Slaughtering for beef is allowed 14 days after immunization. There are no restrictions on the use of milk from animals inoculated with STI vaccine.

Veterinary workers, administrators of agricultural agencies, as well as the chairmen of kolkhozes and directors of sovkhozes must inform kolkhoz and sovkhoz workers thoroughly on the essence of anthrax, methods of preventing and controlling it.

Mandatory measures when farm animals are stricken with anthrax: If a disease appears among farm animals suspected of being anthrax, the administrator of the livestock farm (kolkhoz, sovkhoz) or owner of the animal must immediately inform the closest veterinarian or feldsher.

In addition to reporting the disease, the farm manager or owner of the animal also takes immediate steps to prevent the spread of the disease, without waiting for the arrival of the veterinarian or feldsher. In the case of indoor upkeep of animals, when the disease was limited to isolated cases, the sick animals should be transferred into a separate place. The stalls where the sick animals had been, as well as objects that had been in contact with them must be thoroughly cleaned and disinfected.

Upon receiving information about a case of anthrax or death of animals suspected of being stricken with anthrax, the veterinarian or feldsher immediately examine sick or dead animals. Autopsies are performed only in extreme cases, when it is impossible to make the diagnosis by means of examination and microscopic blood tests.

When anthrax is diagnosed, all of the cattle at the farm (herd, separate yards, etc.) must have their temperature taken, and animals that are sick or suspected of being sick are put in separate groups.

Upon making the diagnosis of anthrax, the veterinarian or feldsher with the partipation of the farm manager, organize cleaning and disinfection of the area and buildings where sick animals or carcasses of dead animals had been, and concurrently determine the causes and source of the disease, taking steps to eliminate them, giving instructions pertaining to a change in pastures for cattle, among which there had been cases of anthrax.

The veterinarian or feldsher immediately inform neighboring district veterinarians, local public health agencies and the chief physicians of the rayon department of agriculture about the appearance of anthrax among animals, and the latter immediately informs the ispolkom of the rayon council of workers' deputies, the veterinary administration of the republic's ministry of agriculture, veterinary department of the oblast (kray) administration of agriculture, as well as the commanding officers of military units [chasti], if any are located on the involved territory.

Populated centers, as well as sections, herds, flocks, etc., in which there have been cases of anthrax among animals, must be quarantined. The quarantine is established by decision of the ispolkom of the local council of workers' deputies.

The quarantine conditions forbid transporting or driving animals through the quarantined area, transporting or driving animals into or out of it, as well as removal from the quarantined farm of fodder, milk, dairy products, hides, wool, hair, hooves, etc.

The following are forbidden within the quarantined territory: use of milk from sick animals and slaughtering animals for meat; 2) dissection of animal carcasses and removing hides from dead animals; 3) regrouping, exchange and sale of animals; 4) performance of surgery, with the exception of emergencies; 5) organization of markets, fairs, exhibitions of animals, as well as other activities involving congregation of animals.

Animals with anthrax or suspected of having this disease are treated. All other susceptible animals at a given farm or part of them are inoculated against anthrax.

Passive immunization with anthrax serum is performed on animals that had been in the immediate vicinity of sick or dead animals.

Special personnel immunized against anthrax are assigned as separate service personnel for the care of anthrax-stricken animals or animals suspected of having this disease in the quarantine centers (farms), and they are furnished with special work clothes.

Fodder contaminated by the excrements of anthrax-stricken animals, manure, litter and leftover feed from sick animals are burned on the spot or near the infected place, with adherence to fire safety rules.

The carcasses of animals that died of anthrax may be used in salvage [heat recovery] plants in autoclaves with pressure of at least 4 atm, where they are kept for at least 4 h. If it is impossible to salvage them in this manner, the carcasses along with the hides must be burned. It is foribidden to bury anthrax-stricken carcasses.

The place in a pasture where the carcass of an anthrax-stricken animal had lain is thoroughly burned out, then covered with 20% calcinated lime containing at least 25% active chlorine, or with 10% hot caustic soda solution, after which the soil is dug up and again profusely covered with the above disinfectants, and mixed with dry calcinated lime at the rate of one part lime to three parts soil.

The milk from anthrax-stricken animals and those suspected of having anthrax is destroyed. Milk from other animals in quarantine is used only after being boiled.

The quarantine is lifted 15 days after the last case of death or recovery of an anthrax-stricken animal. The final measures include thorough mechanical cleaning of the building for the livestock and adjacent territory, and final disinfection (Table 9).

When anthrax-stricken animals or suspected ones are found among animals delivered for shipment over the railroad or in transit, the entire batch of cattle is detained. The animals are submitted to a thorough veterinary examination and their temperature is taken. Sick and suspicious animals are separated from the rest and treated, after which they are sent to the isolation department of the closest rayon veterinary hospital (of the veterinary district). All the other animals in the same group are submitted to passive immunization and quarantined.

The carcasses of dead animals are burned. Pastures, loading areas, platforms, corrals, etc., are disinfected; fodder, inexpensive upkeep objects, litter and manure are burned.

If anthrax is suspected, the veterinarian of the shop (slaughterhouse) must immediately stop the operation of the shop for primary processing and disinfect the livestock-receiving base, livestock buildings, stockyards and equipment.

The carcass of a suspect animal is kept until the diagnosis is made, and if confirmed it is destroyed.

It is allowed to slaughter the animals only after taking all steps to guarantee eradication of infection.

Table 9. Disinfection methods used in areas (farms) with a permanent anthrax problem or in danger thereof and at meat-packing plants

Objects that must be disinfected	Method of disinfection
Territories and livestock-receiving bases, stockyards where batches of animals were kept, among which anthrax was found; buildings in which sick or suspect animals are and were kept	Disinfection is performed three times, at 1-h intervals, with 20% calcinated lime containing at least 25% active chlorine, or 4% formaldehyde, or hot (55°) 10% caustic soda at the rate of 1 liter/m^2 area each time. After the last treatment, the building is closed up for 3 h and then aired Note: mechanical cleaning is performed after the first disinfection: The top layer of soil, at least 15-20 cm deep, is removed from the stall, mixed with 20% calcium hypochlorite and transported to livestock burial grounds in a watertight box.
Metal objects	Disinfected by roasting or flowing steam, or by 3-fold treatment at 1-h intervals with suspension of calcium hypochlorite
Canvas, felt and cotton objects	Decontaminated by boiling in water for 90 min or in 1% soda for 90 min
Leather objects	Decontaminated by thorough washing with sublimate-carbolic solution followed by drying and greasing with tar or grease
Manure, litter, feed left by sick animals	Manure, litters and leftover feed from sick animals is burned locally. Before removal to the burning site, the manure, litter and leftover feed are moistened in disinfectant solution

Prevention of anthrax in industry: The steps taken to prevent occupational-industrial cases of anthrax start at the place of procurement of raw material of animal origin.

All leather and fur raw material to be procured, which does not originate from the slaughterhouse, must be examined for anthrax using the precipitation test in a veterinary laboratory. If there is a positive reaction for anthrax in at least one pelt in a stack, the entire stack is removed into a special isolation room under the supervision of a veterinary specialist and it is disinfected.

According to the instructions dealing with veterinary-sanitary measures for animals, birds, raw material of animal origin, raw animal products and fodder imported to the USSR, which were approved by the USSR Ministry of Agriculture on 12 April 1958, imported hides and pelts procured in areas with a permanent anthrax problem cannot be accepted. This raw material can be imported in the USSR only if there are veterinary certificates attesting to the fact that it originates from an epizootiologically healthy locality and obtained from slaughtering healthy animals. Hides and pelts obtained from slaughtering healthy animals at slaughtering enterprises (meat packing plants, slaughterhouses) with the veterinary inspection stamp can be imported without examination for anthrax. Raw material of other origin must be inspected for anthrax by the precipitation reaction, and if the reaction is positive importation is forbidden. Wool, goat fur, bristle and horsehair can be imported without bacteriological inspection for anthrax if accompanied by a veterinary certificate.

The employees at enterprises that process raw material of animal origin must have a special locker room on the order of a disinfection center with showers, individual lockers for personal and work clothes, lavatories, soap and towels.

Employees at enterprises dealing with procurement, sorting, storage, transportation and processing of pelts, hides, wool, hair, bristle, bones, etc., are inoculated against anthrax.

Warehouse and production rooms of enterprises dealing with procurement, storage and processing of animal raw material are submitted to preventive disinfection at least twice a year. If raw material is found to be infected with anthracic spores, the premises with the infected raw material, equipment, transportation vehicles, etc., are immediately disinfected. The infected raw material must be decontaminated under the supervision of the veterinary inspectorate; if it is impossible to decontaminate it, it must be burned.

Preventive and final disinfection of premises is performed with a solution of calcinated lime containing 25% active chlorine, or 10% hot (75-80°) caustic soda, or 4% formaldehyde solution. Clarified solutions of calcinated lime containing 25% active chlorine are used to disinfect equipment that is not damaged by chlorine. A rag soaked in 4% formaldehyde solution is used to wipe equipment with metal parts, as well as drive belts. Drive belts and equipment that should not be wet are wiped with a rag moistened with a formalin and turpentine emulsion. The used rags are burned.

Surface and waste water removed from the territory of enterprises dealing with processing of animal raw material is disinfected as follows: 1) by boiling the water together with sediment (in a closed container with narrow hole in the lid for steam to escape) for 2 h, or, 2) mixing water (without allowing it to stand and coagulate) with dry calcinated lime containing 25% active chlorine at the rate of 4 parts water for 1 part calcinated lime, with exposure for 6 h, or, 3) calcinated lime solution at the rate of 2 g active chlorine per liter of water with 6-h exposure. Before this, the industrial waste should be cleared of suspended particles by means of coagulation and then allowing it to stand.

The dosage of calcium hypochlorite for disinfection of industrial waste is determined by the agencies of medical-sanitary inspection for each enterprise individually, depending on the physicochemical composition of such water. Disinfected water can be disposed of into the general sewer system. In the absence of a sewer system, the water should be transported in closed containers to places specified by the agencies of sanitary inspection.

Solid waste from raw material, trash, etc., are hauled to specially assigned places and burned. The waste and refuse are packed in waterproof boxes, at the bottom of which one pours 20% calcinated lime (1-2 cm layer) before packing.

Anthracic hides are disinfected by pickling in a solution of hydrochloric acid and table salt, or acidulated sodium fluosilicate.

Pelts preserved by the dry-saltfree and dry-salt methods, with superficial contamination or suspected contamination by anthracic spores are disinfected in a solution of hydrochloric acid and table salt, after preliminary soaking in sour bread drench. Disinfection lasts 40 h at a temperature of 80°. Pelts preserved by the acid method are decontaminated without preliminary drenching.

Wool, bristle and hair raw material and goat fur exposed to anthrax are disinfected in flowing steam chambers at a pressure of 0.5 atm on the pressure gauge (temperature of 111-112°).

POSSIBLE USE OF B. ANTHRACIS FOR BACTERIOLOGICAL WARFARE

For centuries, many scientists of the world--physicians, biologists, chemists--who devoted their lives to research in order to find the causes of communicable diseases, ways and means of diagnostics, therapy and specific prophylaxis, were governed exclusively by humanitarian goals, to save mankind from devastating epidemics. At the early stage of discovery of etiological factors of communicable diseases, development and implementation of control measures, the experiments inevitably had some victims. In a number of cases, researchers conducted experiments on themselves, or died of the pathogen that they were studying, in order to obtain reliable facts.

By using and refining methods of preventing infectious diseases and controlling them, different nations were rid of many communical diseases at different stages of history. In this respect, the results of the 50-year struggle of Soviet medical figures are the most striking; they were able not only to eradicate, within a relatively shorter time, the serious epidemic legacy of tsarist Russia, depleted by imperialistic intervention and civil war, but to achieve results that the most developed capitalistic nations of the world do not have.

The socialist public health system founded by the communist party of the Soviet Union makes it possible to use the most effective and rational methods of controlling infectious diseases and preventing them, implementation of which over the entire nation is not restricted by financial resources.

The natural process of studying the pathogens of communicable diseases in order to develop effective ways and means of preventing them at the start of our century and especially in the 1940's in some capitalistic countries became antinatural, deformed. All of the scientific advances in the area of studying viruses and bacteria, and subsequent development thereof in imperalistic Japan and fascist Germany, and later in the United States as well, were directed toward development of biological (bacteriological) warfare. The greatest discoveries of our times, which made it possible to produce large amounts of bacterial mass (deep-layer method of cultivation), to use various cellular systems to accumulate viruses, to alter the genetic code of viruses and bacteria are now subordinated to the goals of developing and producing bacteriological weapons in the world's largest capitalistic country, the United States. This research, as validly observed by the well-known English journalist, Robin Clarke, is a negation of everything that has already been achieved by physicians, a negation of medicine itself.

Having approved a program for development of bacteriological weapons during World War II in response to the possibility that fascist Germany and imperalistic Japan could use such weapons, the ruling circles of the United States not only failed to stop this program at the end of the war, but with each year expanded the research and funded it generously. They buried in oblivion the statement made by Roosevelt in 1943, that "no matter what the circumstances, we shall not resort to the use of such weapons unless the enemy uses them first," having formulated in the field service regulations of 1956, which is the guide for the army of the United States, a new statute to the effect that "the United States are not participants of any agreement that is in force at the present time and in which the use of toxic or nontoxic gases or fumes, or flammable substances, or bacteriological warfare is banned or restricted."

Indeed, although the United States did sign the Geneva treaty of 1925, which banned the use of chemical and bacteriological warfare, it did not ratify it. According to the field service regulations, as Hersh writes (1968): "the decision that the Armed Forces of the United States use chemical and bacteriological warfare has to be made by the president of the United States."

In view of the level of scientific and technological advances in many countries of the world and, first of all, the United States, the possibility of developing bacteriological weapons as a means of wholesale destruction should be considered very real. However, the probability of use thereof is determined not only by the aggressive and misanthropic policy of the military circles of the United States, but the breadth of the movement that involves the people of many nations of the world who are against the launching of a new war and for the ban of use of nuclear, chemical and bacteriological weapons in such a war. This probability also depends on the extent to which the people of peace-loving countries will have effective means of defense, capable of neutralizing the hypothetical strike force of such weapons. In-depth knowledge about the pathogens of diseases, particularly those that are the most likely to be used for warfare, is the most important element determining the feasibility of development and use of defense means.

B. anthracis is mentioned among the pathogens that foreign military specialists consider the most promising. This is the only microorganism whose features leave no room for debate as to the possibility of using it.

If we consider that the American researchers concerned with development of bacteriological warfare prefer scarce or seldom encountered pathogens, B. anthracis must be relegated to expressly this group, since most physicians in countries that rid themselves of anthrax know little about this infection.

The probability of using B. anthracis as a bacteriological weapon is mentioned, in particular, by Fox (1942), Rosebury, Kabat, Boldt (1947), Wiesmann (1952) and Kliewe (1954). The numerous cases of intralaboratory infection with this pathogen at Camp Detrik are direct proof of the intensive research conducted on this microorganism in the United States. In only 3 years (1943-1946), 25 cases of anthrax were recorded in this bacteriological research center (Feiner, quoted from V. A. Lebedinskiy, 1960).

What are the distinctive features of B. anthracis that make it possible to use it as a bacteriological weapon?

1. The pathogen of anthrax is very dangerous to man. In the case of spontaneous infection, the disease has a serious course, even in its mildest clinical form,

cutaneous anthrax. As for pulmonary or intestinal anthrax, it is usually fatal. The rapid course of these clinical forms of the disease, which are characterized by serious manifestations, deprive the physician of the possibility of making the correct diagnosis at the early stage, and this, in turn, rules out the use of specific therapeutic measures. It can be stated, without exaggeration (and this applies not only to Soviet public health practice), that the diagnosis of intestinal or pulmonary anthrax is a pathoanatomical diagnosis, which is made at autopsy. We do not know of a clinician who was able to diagnose these forms of the disease while the patient was alive.

2. When evaluating the possibility of using some pathogen as a weapon, foreign specialists attribute special significance to the efficacy of immunoprophylaxis upon infection with such a pathogen.

The opinion is widely held that the use of B. anthracis antigens for inoculation, in particular live vaccine, could produce stable specific resistance in man must be critically evaluated for two reasons. In the first place, this conviction is based chiefly on experience in prevention of anthrax among farm animals or on experimental data involving the use of such animals, whereas simple extrapolation of the patterns of immunogenesis observed in animals to man is hardly justified. In the second place, all practical immunoprophylaxis, the objective of which is to provide specific protection to man against the natural routes of infection and which applies to the possible infective doses via such routes, cannot of course answer the question of efficacy of one vaccine or other in the case of unusual routes of infection by massive doses of pathogen.

3. B. anthracis spores are exceptionally resistant to various deleterious environmental factors. They remain viable for a long time when heated and boiled, and they are killed only by sterilization at high pressure and high (over 100°) temperature. Solar radiation does not have a devastating effect on them, while the usual disinfectants used to decontaminate environmental objects from the vegetative forms of microorganisms do not affect spores.

4. The resistance of B. anthracis spores makes it possible for them to accumulate in unlimited quantities, to store them for a long time, to dry them under conditions that are devastating to vegetative forms of microorganisms and to use them for military purposes by different methods. The very fact that there are endemic sites of this infection in nature, due to the prolonged viability, lasting for decades, of spores in soil warrants stating that contamination of environmental objects with B. anthracis spores, when they are used as a bacteriological weapon, could create conditions hazardous to human life for a long time, and would require exceptional efforts to disinfect these objects.

5. Unlike many microorganisms, B. anthracis grows well and profusely on readily available synthetic nutrient media. As shown by the experience of vaccine production, the deep-layer method of cultivation in large containers can be used. In this regard, there is a logical assumption that imperialistic countries, first of all the United States, who have taken the route of developing bacteriological weapons, are able to make use of the production capacity of the antibiotic industry and that of other biological products to produce B. anthracis spores.

In 1968, the book by Robin Clarke, entitled "Silent Weapons," describing development of chemical and biological warfare in the United States, was published there. It is not by chance that the author gave this title to his book stressing that bacteriological weapons, being a weapon of wholesale destruction and intended primarily to destroy the civilian population, could be used surreptitiously and suddenly, by spraying bacterial suspensions over large areas in order to infect the atmospheric layer near the ground. Without going into the technical aspects of producing bacterial aerosol in the near-earth atmosphere, which is not within the competence of these authors, we must merely stress that the use of B. anthracis spores as a bacterial aerosol could cause immediate infection of unprotected people, which will result in mass scale occurrence of severe clinical forms of the infection. At the same time, after settling on the surface of environmental objects, B. anthracis spores will create long-term secondary infection sites, where man can become infected via the alimentary route (infection of products and water) and due to penetration of pathogens through the injured integument.

On the basis of historical data and hypotheses, some foreign authors think there is a possibility of using B. anthracis spores in other ways. It is known that, in 1916, agents in Germany under the rule of the Kaiser tried to use B. anthracis spores for diversionary infection of horses and cattle in Romania (Roznjatowski, Zoltowski, 1959). In 1918, the French government was compelled to stop the export of cattle from Argentina, since cases of infection with B. anthracis and glanders by German diversionists became known among the cattle purchased by the allies in Latin America (V. A. Lebedinskiy, 1960). The Japanese specialist, Sakaki [illegible]khei, thinks it is possible to use B. anthracis spores in shrapnel shells. In his opinion, these shells could be used with success to fire on enemy positions, since those wounded by shrapnel have serious complications in the form of anthrax (Knobloch, 1955). According to Wiesmann et al. (1952), some foreign bacteriological warfare theoreticians do not rule out the use of B. anthracis spores for diversionary infection of people and farm animals via the alimentary and contact routes. They believe it is possible to artificially spread anthrax among domestic animals and by means of blood-sucking vectors, for example, horseflies, in whose stomatic system the pathogen of anthrax can survive for up to 5 days.

We could go on with the description of analogous statements, which are widely published in the foreign press and, unfortunately, often taken as the truth. But they do not have a direct bearing on bacteriological weapons. Any diversion, including those using bacterial products, can inflict some damage, being merely local in nature. The actual definition of bacteriological weapons as a wholesale destruction weapon implies use of bacterial agents on a mass scale over large areas, in major industrial and administrative centers, which is feasible only with the use of modern equipment to deliver bacteriological weapons to the target, for infection of the near-earth layer of the atmosphere.

Presentation of only the "strong points" of bacteriological warfare could cause the reader to feel frightened and doomed, and this is what the American ruling circles are counting on. They present all of the achievements in the area of development of bacteriological warfare in a sensational manner, stressing its hazard and remaining silent about the possible effective means of protection. The journal, SHPIGEL' (No 52, 1967), commenting on a speech of former president Johnson of the United States dealing with the achievements of a team of scientists headed by Arthur Kornberg, who synthesized deoxyribonucleic acid, which opens up the possibility of producing new viruses, reported that the "California medical scientists are one step closer to fulfillment of their cherished dream and nightmare that could threaten mankind: now the scientists could develop the most horrible weapon, artificial pathogens, against which there are no vaccines and no medical protection."

First of all, it must be noted that bacteriological warfare is uncontrollable to a significant extent. The effectiveness thereof depends on meteorological conditions and time of day. As a weapon of wholesale destruction, it can be used only in aerosol form, for which reason all of the physical laws of behavior of aerosols in the atmosphere apply to bacteriological weapons. The physical stability of bacterial aerosol is determined by the ascending and descending currents of wind, while its biological activity depends on how long the bacterial particles remain under specific conditions of atmospheric air and degree of sensitivity of the microorganism to exogenous factors (humidity, insolation).

The extent of our medical knowledge in the field of pathology, vast experience in eradicating and lowering the incidence of many infectious diseases, the existing system of epidemic control, the basis of which is created by specialists who make use of the latest advances in Soviet and foreign science--all this makes it possible to strengthen and refine antibacterial protection of the nation.

Antibacterial protection as a system of measures is not a synonym for epidemic control work. The latter is a component of antibacterial protection, an important element thereof, which is used both for the prevention of possible disease (immunization prior to use of bacteriological warfare) and eradication of the sequelae of such use.

Knowledge about infections, diagnostics, symptomatology, therapy and prevention thereof are the most important elements of antibacterial protection, which determine its efficacy. Anthrax is one of the infections that merits exceptional attention in the light of the foregoing.

Antibacterial protection as a system of measures can be divided into two main sections: measures that preclude the possibility of using biological (bacteriological) agents for military purposes, and measures to eliminate the sequelae of such use. Of course, the first part includes not only and not so much medical aspects of the problem. The latter amount to critical analysis of information published abroad and serious training of medical personnel in the area of infectious pathology of man on a wide scale, scientific development of rational systems and methods of immunoprophylaxis against particularly dangerous infections, and practical implementation of these measures in peacetime. In view of the fact that the efficacy of any weapon, including bacteriological ones, is determined not only by its distinctions, but level of preparedness to defend itself against the pathogen of the country that has become the victim of such an attack, it can be maintained that serious preparation for defense against bacteriological warfare will dampen the ardor of aggressive imperialistic circles, which have taken the road of development of such weapons of wholesale destruction. It is quite obvious that one must give special attention to deployment and intensification of a campaign among the people of the world to condemn and ban biological warfare.

Among the measures for protection against bacteriological weapons, which are designed to avert possible infection at the time bacteriological warfare is used, we should single out, first of all, the general, nonspecific defense measures. They are the same, for the protection of man against both chemical and bacteriological warfare. We refer to gas masks and sealed shelters, to which filtered air is delivered. Even the ordinary sealing of windows and doors could prevent, to a significant extent, penetration of bacterial aerosol into a dwelling. But the use of nonspecific identification ["indication"] of bacterial aerosol in atmospheric air over wide areas and automatic reporting of danger are a mandatory prerequisite for the effective use of such measures.

As for specific means of protection, production of strong immunity in most of the public before the use of bacterial weapons would be a complete guarantee of protection. This prospect, as it applies to the pathogen of anthrax, is based on the existing experimental data. Like any mass scale immunization measure, it is complicated with regard to administration [organization], but quite feasible with the existing system of prevention and control of infectious diseases inherent in the Soviet public health service.

Specific identification of the pathogen is the basis of bacteriological control measures to eradicate the sequelae of a bacteriological attack. The earliest possible identification of the pathogen used as a weapon, measurable in hours, makes the following feasible: 1) immediate emergency prophylaxis prior to appearance of signs of the disease; 2) initiation of early specific therapy, which determines the outcome of illness in the case of anthrax; 3) implementation of measures to localize the region involved (prevention of spread of pathogen to adjacent territories) and disinfection measures on a broad scale, ruling out the possibility of secondary infection due to use of infected clothing, water, food and other environmental objects.

It is quite obvious that the use of ordinary methods of identifying a pathogen, which are based on performance of biological tests on laboratory animals, has no bearing on the term "indication," since the results of such a study would not be obtained after the first cases of death occur from the intestinal or pulmonary form of anthrax.

It must be noted that the marked resistance of B. anthracis spores to deleterious factors, particularly high temperatures that are devastating to vegetative forms of microorganisms, alleviates significantly the job of bacteriological laboratory examination, since it is possible to remove extraneous microorganisms from the specimen that is examined.

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